PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant No. 09/936,872

Title: "FLUORESCENCE ELETRONIC ENDOSCOPIC SYSTEM"

U.S. Filing Date: September 17, 2001

Reply to the action filed on June 16, 2005

Eleni Mantis Mercader
Primary Examiner
Art Unit 3737

Sir:

I, the applicant, on the ground of newly submitted references, strongly protest against the claim rejections.

The reason the examiner raised to reject the claim was that experts who had the knowledge taught by MacAulay et al. (United States Patent Number 5,590,660. Filed: Mar. 28, 1994) and Wagnieres et al could easily develop the same invention as mine. But, the newly-submitted references described later clearly prove that this is completely not correct.

In 1998, Lam et al. (Reference 1) published the results of an initial trial performed with the laser-induced fluorescence endoscopy (LIFE) system (Xillix Technologies, Vancouver, Canada) (which is the invention of MacAulay et al.) in 173 patients with suspected lung cancer in whom white light bronchoscopy was followed by autofluorescence (AF) bronchoscopy. However, researchers found out that there were some significant problems of the LIFE systems.

1) LIFE employs a helium-cadmium laser light source, with two image intensifier CCD cameras and a color video monitor for imaging. **The system is somewhat bulky and, due to the two cameras, it does not allow direct and immediate comparison of white light and AF image** (Reference 2, page 396).
2) LIFE has difficulty distinguishing between preinvasive lesions and other benign epithelial changes such as bronchitis, which frequently is present in patients whose sputum cytology is suspicious of malignancy or positive for malignancy (Reference 3, page 308). The specificity of fluorescence bronchoscopy is reported to be rather low with up to one-third of the areas of abnormal fluorescence representing false positive. And it is noted that **this low specificity leads to unnecessary biopsies, resulting in greater economic cost and longer examination time** (Reference 3, page 308).
3) In a randomized cross-over study, no increase in the rate of detection of high-grade dysplasia or early cancer was found in Barrett's esophagus (BE) when using LIFE. There are two possible explanations for these results. **First, in the currently available prototypes, the algorithm used to construct the AF image may not be optimal for BE; it only accounts for the ratio of red to green AF and does not incorporate information from reflected light. The LIFE systems studied included fiberoptic endoscopes, which provide a poor white-light image compared with currently available, high-quality videoendoscopes (Reference 4, page 679).**

(It should be emphasized here that from the above facts it becomes evident that based on the knowledge taught by MacAulay et al. it is practically impossible to integrate the fluorescence image with images provided by reflectance light even in the year 2005 when Reference 4 was published.)

In order to solve these problems, newer AF systems have been introduced which are cheaper and more convenient in their handling. These include D-light system (Storz, Tuttlingen-Germany) (Reference 5) and the SAFE 1000 (References 6-7) autofluorescence systems (Pentax, Asahi Optical Tokyo, Japan)

D-light system

The D-light system uses a 300-watt xenon lamp, which is optimized for blue light transmission. In the AF mode, wavelengths form 380 to 460 nm are used for excitation. For detection, a filter integrated in the eyepiece of the bronchoscope blocks most of the excitation light. Light intensity and quality of image enable investigation under direct AF guidance. A single small camera enables the white light and fluorescence images, which can be directly observed under dimmed daylight (Reference 2, page 397). In combination with some reflectance of blue light from the tissue, the D-light produces bluish images for normal areas and darkened images in case of malignancies (Reference 8, page 160).

SAFE 1000 autofluorescence system

In this system, excitation uses a Xenon-lamp (wavelengths from 420 to 480 nm) and the camera contains a fluorescence filter as well as an image intensifier (References 6; United States Patent Number: 5,891,016. Filed: Oct. 30, 1996), (References 7 – 8).

In 2001 Homasson et al. denoted that LIFE, using a laser helium-cadmium excitation source, was expensive and bulky. In contrast, SAFE 1000 and D-light systems without using laser illumintion are easy to use. Lower cost would allow more widespread use of these systems currently reserved for risk populations (Reference 9, abstract). In 2003, Herth et al. (Reference 2) compared two fluorescence-imaging systems, LIFE (Xillix Technologies, Vancouver, Canada) versus the D-light system (Storz, Tuttlingen-Germany; actually a fluorescence-reflectance system) in 332 patients at risk. Both systems yielded comparable results, i.e. no significant differences in finding or missing (pre-)neoplastic lesions, and the D-light is easier to handle, leading to a shorter average examnination time of 8 versus 12 min for LIFE (Reference 2, page 395; Reference 10, page 347).

However, it was considered that the SAFE-1000 and the D-light systems were still not good enough. As LIFE, these systems use a fiberscope in combination with CCD camera (References 5-7). The

SAFE-1000 provides only dim fluorescence images as LIFE (Reference 3, page 310, figure 3; Reference 2, page 398, figure 1). The image of the D-light system, although using combination with some reflectance of blue light from the tissue, is still quite dim (Reference 2, page 398, figure 2). The SAFE-1000 and the D-light systems have also a low specificity for diagnosing preinvasive lesions of the lung as LIFE does (Reference 3, page 312).

In order to cover the disadvantage of dim fluorescence image, Pentax (Asahi Optical Tokyo, Japan) has developed a new fluorescence diagnosis endoscope system (Reference 11; United States Patent Number: 6,099,466. Filed: Nov. 10, 1997) able to provide simultaneously the fluorescence image and white light image. A pair of image receiving elements are placed at the tip of an endoscope; one with a fluorescence filter to provide fluorescence image and the other monochromatic one without any filter to provide white light image. **It is noteworthy that in this system a rotary red/green/blue band-pass filter is used to provide white light image. However, the inventors had no idea that they could obtain an integrated image of the fluorescence image and images provided by reflectance lights just by placing the fluorescence filter in front of the monochromatic image receiving element.**

The idea to integrate the fluorescence image with the remittance light image did not appear in United States Patent Number: 6,280,378 filed: May 28, 1999, either (Reference 12). As United States Patent Number: 6,099,466, fluorescence image and white light image are simultaneously shown on separated screens. Compared to LIFE, the SAFE-1000 and the D-light systems, United States Patent Numbers: 6,099,466 and 6,280,378 made a progress by using videoscope instead of fiberscope and using charge-coupled devices (CCD) instead of bulky CCD camera. However, that the fluorescence image is quite dim cannot be resolved by these inventions, because they do not integrate the fluorescence image with the remittance light image.

In 2005 there were reports (References 3-4) on a newly developed prototype autofluorescence imaging videoscope system provided by

Olympus Optic Co, Ltd, Tokyo, Japan. In this system, a high-resolution videoendoscope is used to produce high-quality white light images. Moreover, a different algorithm (from that of LIFE) is used to construct an autofluorescence (AF) image (Reference 4, page 680). This system has a xenon light source (XCLV-260HP; Olympus), with a rotary red/green/blue band-pass filter. With this light source, the mucosa is sequentially illuminated with red, green, and blue light at a frequency of 20 cycles per second. The high-resolution videoendoscope in this system has two separate monochromatic charge-coupled devices (CCD); one for white-light endoscopy (WLE), and one for AFI. In the WLE mode, the reflected red, green, and blue light is detected by the standard CCD and is converted to an electronic signal that is passed to the videoprocessor, which is synchronized with the rotary filter. The processor electronically overlays the red, green, and blue signals to produce a high-quality white-light image. In the AFI mode, blue spectrum light (wavelengths from 395 to 475 nm) is delivered for excitation of AF, together with light in the green (wavelengths from 540 to 560 nm) and the red (wavelengths from 600 to 620 nm) spectra. The AFI-CCD has a barrier filter that allows detection of all light with a wavelength between 490 and 625 nm, thereby eliminating the blue excitation light. The sequentially detected images from AF, green reflectance, and red reflectance are integrated by the image processor into one AF image. The AF image, therefore, has 3 spectral components: (1) total autofluorescence in response to blue light excitation, (2) green reflectance light, and (3) red reflectance light (Reference 4, page 680). The AF images obtained are brighter and of higher resolution than those of LIFE (Reference 3, page 310, figure 3; Reference 4, page 681, figures 1 and 2). The specificity in detecting lung preinvasive lesion by this newly developed autofluorescence imaging bronchovideoscope system (83.3%) was significantly higher than that of LIFE (36.6%) (p=0.0005). It was concluded that autofluorescence imaging bronchovideoscope system appears to represent a significant advance in distinguishing preinvasive and malignant lesions from bronchitis or hyperplasia under circumstances where LIFE would identify these all as abnormal lesions (Reference 3, pages 307-308). It was also concluded that this autofluorescence imaging videoendoscope system may improve the detection of high grade dysplasia/early cancer in

patients with Barrett's esophagus (Reference 4, page 679).

Note that the algorithm used in this newly developed autofluorescence imaging videoscope system is exactly the same as that of my invention (Reference 13, page 567-569). In my paper published in the year 2002, it has already been noted that my fluorescence electronic endoscopic system could be used to observe autofluorescence also (Reference 13, page 567, figure 8F). There is only minor difference in displaying the images. In my paper, the autofluorescence image is displayed as blue, the green reflectance light image as green and the red reflectance light image as red (Reference 13, page 570, figure 13). In References 3 and 4, the autofluorescence image is displayed as green, the green reflectance light image as red and the red reflectance light image as blue (Reference 3, page 309). I, therefore, consider that Refernces 3 and 4 are the evidences showing violations of my Japanese patent by Olympus.

From the References mentioned above, it becomes evident that experts in endoscopic field (Reference 4, page 680) accept that my invention uses different algorithm from that of LIFE developed based on the knowledge taught by MacAulay et al. (United States Patent Number 5,590,660. Filed: Mar. 28, 1994), and that my system is superior to LIFE in all aspects: much brighter and higher resolution image, much easier to operate and much cheaper. Moreover, the specificity in detecting preinvasive lesions is significantly higher (Reference 3, pages 307-308). I, therefore, see no reasons why my invention should not deserve the United States Patent. It would be really ridiculous if the examiner who is not an expert would still consider that it was easy to develop the same invention as mine with the knowledge taught by MacAulay et al. and the knowledge of using a monochromatic CCD together with a rotary red/green/blue band-pass filter in videoendoscope (this type of videoendoscope has been used around the world for more than 15 years) (References 14; United States Patent Number: 4,870,487. Filed: Sep. 15, 1988). If it was easy to do so, it should not have taken about 7 years since the development of LIFE system (Reference 1) for Olympus to develop prototype video autofluorescence endoscopy system with the same algorithm as mine (References 3-4); after the development of LIFE, Storz should never have developed the D-light system and Pentax should

never have developed the SAFE-1000, both of which are still much inferior to my invention. The history of developing autofluorescence endoscopy system (References 1-12) proves by itself that it was not easy for endoscopic experts of Xillix Technologies, Storz, Pentax, Fujinon and Olympus to develop the same invention as mine.

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REMARKS

If there are any fees required by this communication, please inform the applicant at the fax phone number –81-29-851-3721.

Respectfully submitted,

Date: August 16, 2005

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CERTIFICATION OF MAILING

I hereby certify that this correspondence is being deposited with EMS mail in an envelope addressed to Eleni Mantis Mercader, Primary Examiner, Art Unit 3737, UNITED STATES PATENT AND TRADEMARK OFFICE, P.O. Box 1450, Alexandria, VA. 22313-1450, on August 16, 2005.

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Date of Sig.: August 16, 2005

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REFERENCE 1

Localization of Bronchial Intraepithelial Neoplastic Lesions by Fluorescence Bronchoscopy*

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Background: **In the treatment of lung cancer, the best outcome is achieved when the lesion is discovered in the intraepithelial (preinvasive) stage. However, intraepithelial neoplastic lesions are difficult to localize by conventional white-light bronchoscopy (WLB).**

Objective: **To determine if autofluorescence bronchoscopy, when used as an adjunct to WLB, could improve the bronchoscopist's ability to locate and remove biopsy specimens from areas suspicious of intraepithelial neoplasia as compared with WLB alone.**

Method: **A multicenter clinical trial was conducted in seven institutions in the United States and Canada. WLB followed by fluorescence examination with the light-induced fluorescence endoscopy (LIFE) device was performed in 173 subjects known or suspected to have lung cancer. Biopsy specimens were taken from all areas suspicious of moderate dysplasia or worse on WLB and/or LIFE examination. In addition, random biopsy specimens were also taken from other parts of the bronchial tree.**

Results: **The relative sensitivity of WLB+LIFE vs WLB alone was 6.3 for intraepithelial neoplastic lesions and 2.71 when invasive carcinomas were also included. The positive predictive value was 0.33 and 0.39 and the negative predictive value was 0.89 and 0.83, respectively, for WLB+LIFE and WLB alone.**

Conclusion: **Autofluorescence bronchoscopy, when used as an adjunct to standard WLB, enhances the bronchoscopist's ability to localize small neoplastic lesions, especially intraepithelial lesions that may have significant implication in the management of lung cancer in the future.**

(CHEST 1998; 113:696-702)

Key words: autofluorescence; bronchoscopy; early detection; lung neoplasm

Abbreviations: CI=confidence interval; LIFE=light-induced fluorescence endoscopy; WLB=white-light bronchoscopy

Lung cancer is the most common cause of cancer deaths in North America. Despite advances in the detection and treatment of many cancers leading to improvements in the 5-year survival rates, the survival rate for lung cancer continues to be <15%.[1] The main reason for the continued low survival rate for lung cancer patients is that tumors are found at a late invasive stage, when the options for treatment are mostly palliative. Experience in other epithelial organs such as the cervix, esophagus, and colon has shown that if the neoplastic lesions can be detected and treated in the intraepithelial stage, the cure rate can be improved significantly.[2-4] Unfortunately, in the tracheobronchial tree, although a number of endobronchial treatment modalities have been developed for the curative treatment of small intraepithelial (preinvasive) neoplastic lesions,[5-8] identifying individuals harboring these lesions and localizing their exact sites remain problematic. For example, in a study by Woolner,[9] only 29% of the carcinoma *in situ* detected by sputum cytology examination could be localized by conventional white-light bronchoscopy (WLB).

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When the bronchial surface is illuminated by light, the light can be reflected, back-scattered, absorbed, or induce tissue fluorescence. Conventional white-light examination makes use of the first three optical properties—a process that is known as reflectance imaging. The tissue autofluorescence is not visible to the unaided eye because its intensity is very low and overwhelmed by the reflected and back-scattered light. However, with suitable instrumentation, the tissue autofluorescence can be visualized.[10] It was further observed that fluorescence intensity differs significantly between normal and neoplastic tissues and that this difference can be exploited to enhance our ability to localize areas of intraepithelial neoplasia in the tracheobronchial tree.[11-13]

The objective of this study was to determine if fluorescence bronchoscopy using the light-induced fluorescence endoscopy (LIFE) device (Xillix LIFE-Lung Fluorescence Endoscopy System; Xillix Technologies Corp; Richmond, BC, Canada), when used as an adjunct to WLB, could improve the bronchoscopist's ability to locate areas suspicious of intraepithelial neoplasia for biopsy and pathologic examination, as compared with WLB alone. Intraepithelial neoplasia was defined as lesions that were moderate/severe dysplasia or carcinoma *in situ*. These lesions were considered to be important to detect because previous studies showed that approximately 10% of moderate dysplasia and 40 to 83% of severe dysplasia will progress to invasive cancer.[14-16]

Materials and Methods

The clinical trial was conducted at seven institutions: six in the United States and one in Canada. The protocol was approved by the institutional review board at each participating center, and written informed consent was obtained from all patients.

Study Design

Study Population: One hundred eighty-six subjects were evaluated for study participation. The criteria for enrollment in the study were as follows: (1) patients with known or suspected bronchogenic carcinoma who were scheduled for bronchoscopy as part of a standard diagnostic workup; (2) patients with completely resected stage I lung cancer without evidence of metastatic disease; and (3) patients with moderate or higher degree of atypia in their sputum cytologic study with a normal chest radiograph. Patients were excluded from the study if they had received photosensitizing agents such as hematoporphyrin derivative or chemopreventive drugs such as retinoids within 3 months prior to the bronchoscopic procedure, radiotherapy to the chest, or cytotoxic chemotherapy agents within 6 months of the procedure. Other exclusion criteria were known or suspected pneumonia, acute purulent bronchitis, uncontrolled hypertension, unstable angina, bleeding disorders, pregnancy, and known reactions to topical lidocaine (Xylocaine).

One hundred seventy-three subjects met the enrollment criteria. Of these, 114 were suspected to have lung cancer based on their symptoms, smoking history, age, and/or an abnormal chest radiograph; 29 had abnormal sputum cytology findings and a normal chest radiograph; 19 had previous resection for lung cancer; and the remaining 11 were known to have lung cancer from previous investigations.

The bronchoscopic procedure was performed in three stages. First, standard white-light examination was carried out. With the exception of those patients who were to go on to have a thoracotomy, the procedure was carried out under local anesthesia to the upper airways with or without sedation using a fiberoptic bronchoscope (Olympus BF20D; Olympus America Inc; Melville, NY). Normal and abnormal areas as well as areas suspicious of intraepithelial neoplasia or invasive carcinoma were recorded in the image management system of the LIFE device in both digital form and on super VHS video in real time with a video camera. A three-point classification system was used (Table 1). No change in classification in any site was allowed once the white-light examination had been completed. Under this visual classification system, areas without any visual abnormality were classified as class 1. Under white-light examination, areas with nonspecific erythema, swelling or thickening of the bronchial mucosa, bronchoscopic trauma, anatomic anomalies, or granulation tissue were classified as class 2. Nodular/polypoid lesions, irregularity of the bronchial mucosa, or focal thickening of a subcarina suspicious for high-grade dysplasia or cancer were labeled as class 3. Under fluorescence examination, normal areas appear as green while areas suspicious of moderate dysplasia or worse had a definite brown or brownish-red color. The latter was labeled as class 3. Areas that were slightly brown with ill-defined margins that could be easily missed were labeled as class 2. These color changes corresponded to our previous spectroscopic finding that as the tissue changed from normal to hyperplasia, metaplasia, various grades of dysplasia, carcinoma *in situ*, and then to invasive cancer, there was a progressive decrease in the intensity of the emitted fluorescence, especially in the green region of the fluorescence spectrum.[11] The LIFE-lung device was calibrated by the manufacturer to maximize the color difference between the benign or "low-risk" lesions (hyperplasia/metaplasia/mild dysplasia) vs the "high-risk" lesions (moderate dysplasia or higher grade) to reduce the probability of false-positive biopsy specimens.

At the second stage, while the bronchoscope remained in place in the trachea of the patient, the examination was repeated after changing the camera to the fluorescence camera and the light illumination from a white-light xenon lamp to a blue light from a helium-cadmium laser (442-nm wavelength). Details of the LIFE device have been reported previously.[17] During the fluorescence examination, the physician was again required to identify and classify areas of abnormality with the same classification scheme in Table 1.

Following the visual bronchoscopic examinations, in the third stage, biopsy specimens were obtained from all areas graded as

Table 1—*Visual Identification of Normal and Abnormal Bronchoscopic Findings*

Class No.	Description	Findings
1 "Normal"	No visual abnormality	Negative
2 "Abnormal"	Inflammation, trauma, granulation tissue, hyperplasia, metaplasia, mild dysplasia	Negative
3 "Suspicious"	Changes suggestive of moderate/severe dysplasia, carcinoma *in situ*, or invasive cancer	Positive

class 3 by either white light or fluorescence examination. Some of the biopsies were performed under the fluorescence examination to assure proper acquisition of tissue from very small suspicious areas. Two to four biopsy specimens were also taken from areas identified as class 1 and/or 2. The biopsy slides were first evaluated by the local pathologist without knowledge of clinical findings and then all were reviewed by two reference pathologists (J.C.L., A.F.G.) who were blinded to the previous results. The pathologists coded the biopsy slides based on a nine-point scheme (Table 2). The final pathologic diagnosis was determined by a majority decision of the three pathologists. If a final diagnosis could not be reached, the two reference pathologists rereviewed the slides and came to a consensus. Intraepithelial neoplasia was defined as lesions that were graded as moderate/severe dysplasia or carcinoma *in situ* (grades 5 and 6, Table 2).

A biopsy specimen was considered evaluable and included in the study if the bronchial epithelium was intact for interpretation and a matching pair of white-light and fluorescence images of the biopsy site was available for review by the clinical trial monitor.

Statistical Analysis

The physician's bronchoscopic classification was converted from a three-point scale to a two-point scale, such that class 1 and 2 became "negative" (−) and class 3 became "positive" (+) (Table 1). The final pathologic diagnosis was converted from a nine-point scale (Table 2) to a three-point scale; codes 1 to 4 were labeled as "negative" (−); codes 5 to 8 were labeled as "positive" (+); and code 9 was labeled as not evaluable.

Statistically unbiased estimates of sensitivity and specificity were not possible to obtain because serial sections of the entire tracheobronchial tree would need to be examined after the bronchoscopic procedures for these to be defined. However, since the objective of the study was to determine whether the addition of fluorescence (LIFE) examination to WLB was better than WLB alone, the relative sensitivity, or the ratio of the sensitivity of WLB+LIFE as compared with WLB alone, along with the 95% confidence interval (CI), was calculated to evaluate the contribution of the fluorescence examination to detect neoplastic lesions. A relative sensitivity >1 would indicate a significant improvement of WLB+LIFE vs WLB alone. Two other statistically unbiased estimates, the positive predictive value and negative predictive value, were also calculated to evaluate the performance.

The statistical analyses were calculated for all the data combined (moderate dysplasia or worse) as well as subsets of the data, such as moderate/severe dysplasia and carcinoma *in situ* (intraepithelial neoplasia). The data were analyzed on a per lesion basis and a per patient basis. For the per lesion analysis, each biopsy specimen was evaluated and considered positive if the final diagnosis was positive. For the per patient analysis, each patient was considered positive if he or she had at least one positive lesion (biopsy).

Table 2—*Pathology Coding*

Negative		Positive	
Code No.	Description	Code No.	Description
1.0	Normal	5.0	Moderate/severe dysplasia
2.0	Inflammation	6.0	Carcinoma *in situ*
3.0	Hyperplasia/squamous metaplasia	7.0	Microinvasive carcinoma
4.0	Mild dysplasia	8.0	Invasive carcinoma
9.0	Unsatisfactory		

RESULTS

There were 108 men and 65 women with a mean age of 63 years (range, 36 to 87 years). Ninety-three percent of the participants were current or former smokers with mean pack-years (packs per day × number of years smoked) of 54. A total of 864 biopsy specimens were taken. Of these, 164 were not evaluable—111 because the epithelium was lost or mostly lost during the biopsy procedure and 53 because no matching pair of white-light and fluorescence images were recorded for review. The final pathologic diagnosis for the remaining 700 biopsy specimens were as follows: normal in 321; hyperplasia, metaplasia, or mild dysplasia in 237; moderate/severe dysplasia in 93; carcinoma *in situ* in 9; and invasive carcinoma in 40. Thus, a total of 142 biopsy specimens (20%) were graded as moderate dysplasia or worse; this was then used as the standard to determine the relative sensitivity of the bronchoscopic procedures.

On a lesion-by-lesion analysis, WLB alone detected 35 of 142 lesions that were moderate dysplasia or worse. With the addition of fluorescence bronchoscopy, 95 of 142 lesions were localized (Fig 1). The remaining 47 lesions were found by the random biopsy specimens from areas that were classified as 1/2 (21/47 lesions were class 1 and 26/47 lesions were class 2); these lesions were classified as false-negative biopsy specimens. Therefore, the relative sensitivity of WLB+LIFE vs WLB alone was 2.71 (95% CI, 2.13 to 3.70) (Table 3).

On a per patient basis, the sensitivity of WLB for detecting individuals harboring at least one lesion of moderate dysplasia or worse was 37.3%; with the addition of the fluorescence examination, the sensitivity was 75% (Fig 2). Therefore, the relative sensitivity was 2.00 (95% CI, 1.45 to 2.76).

Probably a much more important result of the clinical study is the effectiveness of fluorescence bronchoscopy for localization of intraepithelial lesions (moderate or severe dysplasia and carcinoma *in situ*). Of the 102 intraepithelial lesions, only 9 (8.8%) were localized by WLB alone, whereas the addition of the fluorescence examination identified 57 sites (55.9%). Therefore, the relative sensitivity of WLB+LIFE vs WLB alone for intraepithelial lesions was 6.3 (95% CI, 3.6 to 12.5) (Table 3).

Forty lesions were classified as invasive carcinoma. Twenty-six cancers (65%) were visible by WLB alone. WLB+LIFE identified a total of 38 tumors (95%). The remaining two tumors were not recognized as abnormal by both examinations. They were found by random biopsies of apparently normal areas as control biopsies. Two submucosal tumors were negative on LIFE examination but positive by WLB.

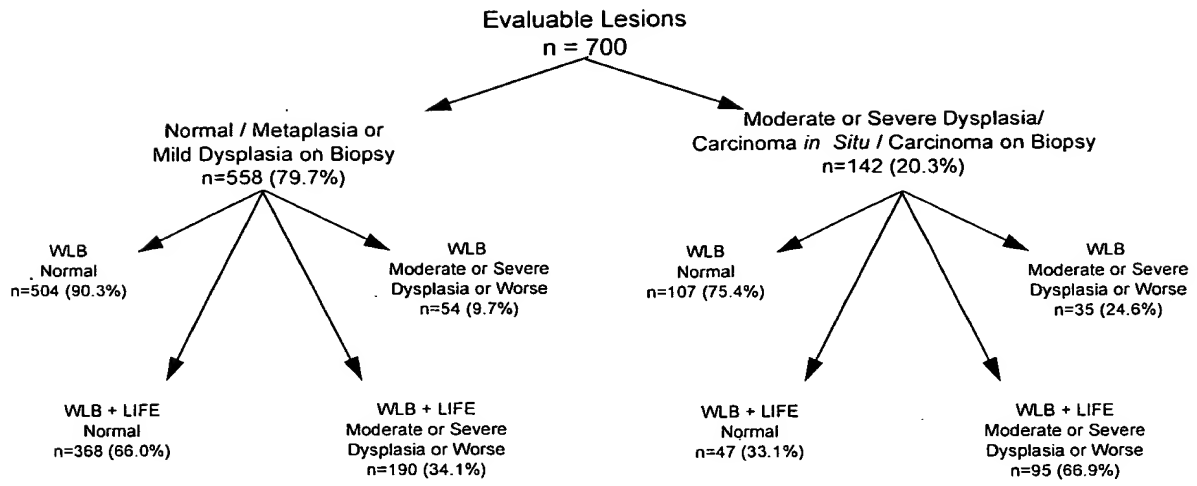

FIGURE 1. Lesion-by-lesion analysis. Results of WLB and WLB+LIFE compared with results on biopsy specimen.

Therefore, the relative sensitivity of WLB+LIFE vs WLB alone for invasive carcinoma was 1.46 (95% CI, 1.21 to 1.95).

Safety

The addition of the fluorescence examination added an average of 13.8 min to a standard WLB, which took an average of 9.4 min. There were no adverse events related to the use of the device.

DISCUSSION

Intraepithelial (preinvasive) neoplasia starts with a molecular phase, in which the epithelium is morphologically normal but is undergoing genomic instability, followed by a morphologic phase, in which aberrant proliferative foci with nuclear and cytologic changes termed dysplasia and carcinoma *in situ* form the basis for the microscopic diagnosis of preinvasive or intraepithelial neoplasia.[18] The concept of carcinogenesis as a multistep process[19] suggests the possibility of blocking or reversing the progression and thus presents opportunities for a more effective intervention in lung cancer. However, intraepithelial neoplastic lesions present a challenging diagnostic problem, even for experienced bronchoscopists. The reason for this is that these lesions are only a few cell layers thick and a few millimeters in surface diameter.[20] Because of this, they may not produce any visible abnormality on conventional WLB. In some cases, subtle changes can be observed in carcinoma *in situ*, consisting of an increase in redness, granularity, or slight thickening of the mucosa. Unfortunately, these changes may also be seen in patients with chronic bronchitis or other inflammatory airway diseases. The lack of progress in the use of conventional WLB for localization of intraepithelial neoplasia is illustrated by two studies.

In a study reported by Cortese and coworkers[21] in 1983, 31.5% of the patients with sputum cytology-positive radiographically occult lung cancer required more than one bronchoscopy for localization. Ten years later, despite a better understanding of the bronchoscopic appearance of early lung cancer, Bechtel and coworkers[22] found that 39% required more than one bronchoscopy for localization of the source of the cancer cells found by sputum cytology examination. This is despite the fact that, on average, only a small proportion of these radiographically occult lung cancers were in the *in situ* stage (33% and 14%, respectively), while the remaining were invasive cancers and hence larger and easier to be visualized bronchoscopically. If a lesion could not be seen, a repeat bronchoscopy with multiple segmental

Table 3—*Summary of Clinical Data per Lesion Analysis**

	Moderate Dysplasia/Worse		Intraepithelial Neoplasia	
	WLB	WLB+LIFE	WLB	WLB+LIFE
Sensitivity	0.25	0.67	0.09	0.56
Positive predictive value	0.39	0.33	0.14	0.23
Negative predictive value	0.83	0.89	0.84	0.89
False-positive rate	0.10	0.34	0.10	0.34
Relative sensitivity	2.71		6.3	

*Intraepithelial neoplasia=moderate/severe dysplasia and carcinoma *in situ*; moderate dysplasia/worse=intraepithelial neoplasia plus invasive carcinoma.

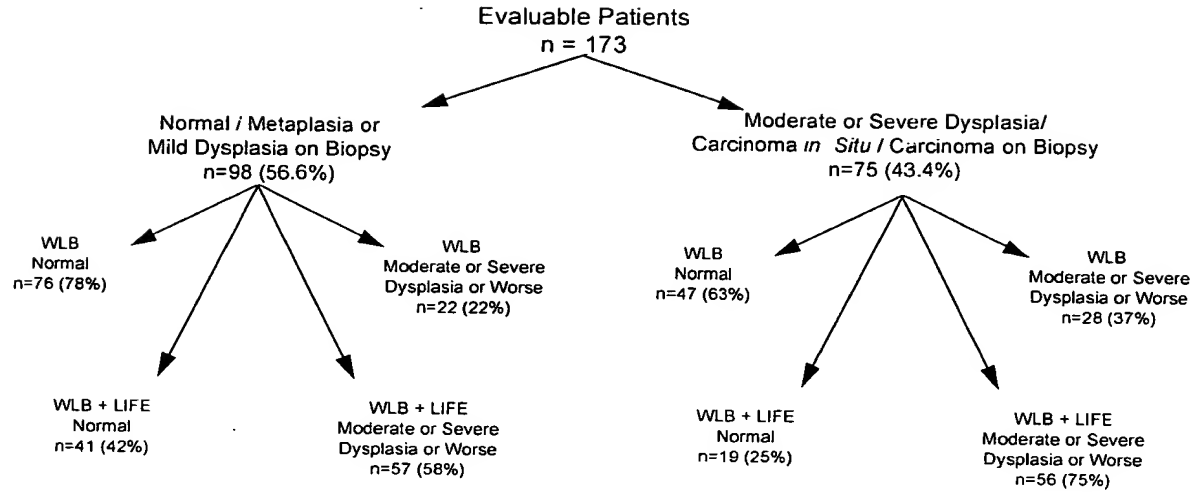

FIGURE 2. Patient-by-patient analysis. Results of WLB and WLB+LIFE compared with results on biopsy specimen.

bronchial brushings and blind spur biopsies were done for localization. Clearly, the task of localizing intraepithelial lesions would be much easier if these lesions could be made visible by other means for direct biopsy.

In the present study, the relative sensitivity of LIFE+WLB vs WLB alone was found to be 6.3 for intraepithelial neoplastic lesions and 2.71 when invasive carcinomas were included. Thus, the addition of fluorescence examination to WLB resulted in the detection of a significant number of early intraepithelial neoplastic lesions that would otherwise be missed. Localization of invasive carcinoma was not the objective of the LIFE examination since most of these lesions are usually visible under WLB. On a per patient basis, the addition of LIFE to WLB doubled (28 to 56) the number of patients classified as positive. However, in this study, we observe that fluorescence bronchoscopy improved the detection rate of invasive carcinoma as well compared with WLB alone.

The reason for the failure of fluorescence bronchoscopy to localize some of the dysplastic lesions found by the random biopsies is not clear at present. Conventional histopathologic study was used as the "gold standard" for comparison with the bronchoscopic findings. Although there is little disagreement in the diagnosis of normal tissue or invasive cancer, significant variation exists among pathologists in the interpretation of intraepithelial lesions.[23,24] Recent advances in quantitative microscopy[25,26] and molecular genetic analysis[27-29] may lead to a more objective classification. Preliminary studies suggest that abnormal tissue autofluorescence correlates better with morphometric measurement and molecular genetic analysis of the bronchial biopsy specimens than with the conventional histopathologic grade (C. MacAulay, AF Gazdar, unpublished data). A recent study showed that only a proportion of the dysplastic lesions expressed matrix metalloproteinases that can degrade the extracellular matrix.[30] Using Monte-Carlo simulation, one of the major causes for the loss of autofluorescence in areas of dysplasia or cancer was found to be a decrease in the extracellular matrix content.[31,32] It remains to be shown whether dysplastic lesions that express extracellular matrix proteinases or those with certain molecular genetic abnormalities are the ones that would progress to invasive cancer. Up until now, there has been no tool that would allow sampling of intraepithelial neoplastic lesions *in vivo*, especially serial sampling of the same area over time to study the pathogenesis of lung cancer and the natural history of these lesions. The availability of fluorescence bronchoscopy has now made it possible for these studies to be carried out to clarify these important issues.[27]

The positive predictive value of LIFE+WLB was 0.33, which was similar to that with WLB alone (0.39). The gross appearance of intraepithelial neoplastic lesions and rarely even invasive cancer can be mimicked by other nonmalignant airway diseases. Although the low positive predictive value is not ideal, the increased sensitivity from the fluorescence examination is a true advantage. The additional biopsies in this study did not result in any adverse events. Furthermore, it is important to recognize that both bronchoscopic examinations simply serve as a guide for biopsy.

Diagnosis and decision regarding clinical intervention are based on the pathologic findings and not the bronchoscopic findings.

Recent advances in defining high-risk groups with a significant incidence of intraepithelial neoplasia,[33] computer-assisted image analysis of exfoliated sputum cells,[25,26] the development of monoclonal antibodies,[34,35] and molecular biology techniques[36] provide promise of a significant improvement in the sensitivity of sputum cytology examination to detect intraepithelial neoplastic lung lesions beyond what has been achieved by the conventional sputum cytology examination used in the screening studies more than two decades ago.[37] However, the improved sensitivity of these newer detection methods may also mean that the size of the underlying bronchial lesions where the exfoliated cells are derived from will be smaller and even more difficult to localize with conventional WLB. Fluorescence bronchoscopy will become an increasingly important clinical tool when these newer sputum examination methods are made available for clinical use.

The major questions that this study opens concern potential therapeutic approaches to these preinvasive and early invasive neoplastic lesions. Preliminary data from several studies strongly suggest that surgery might be avoided in some of the carcinoma *in situ* and microinvasive cancers when treated with photodynamic therapy.[8,38,39] To our knowledge, there have been no other controlled studies using other techniques such as electrocautery, cryotherapy, thermal ablation with the YAG laser, or with brachytherapy since up until now, we do not have any other reliable technique for detection of these lesions in a high-risk population. As far as dysplastic lesions are concerned, this technique would facilitate development of randomized controlled studies for chemoprevention. With the increasing number of chemopreventive agents that are becoming available,[40] sequential sampling of intraepithelial neoplastic lesions using fluorescence bronchoscopy for biomarker studies before and after chemoprevention would allow a more rapid identification of promising agents for population studies.

The technology is now at hand for us to consider developing a new strategy to manage lung cancer better by combining sensitive detection and localization methods with chemoprevention and endobronchial treatment modalities, all of which can be performed in an outpatient setting.

Appendix

The following centers and investigators participated in the LC01 Study Group: British Columbia Cancer Agency and the University of British Columbia, Vancouver, BC, Canada—S. Lam; Pennsylvania Hospital, Philadelphia—M. Unger and M.F. Cunnane; Presbyterian/St. Luke's Cancer Center, Denver Veterans Affairs Medical Center, and the University of Colorado Cancer Center, Denver—T. Kennedy, Y. Miller, R. J. Schwartz, and R. Cook; Kenneth Norris Jr. Cancer Center, Los Angeles—D. Gelmont and R. Barbers; Memorial Sloan-Kettering Cancer Center, New York—V. Rusch; University of California, Los Angeles—B. Gipe, E.C. Holmes, and D. Kelley; Evansville Cancer Center, Evansville, Ind—D. Howard, A. Korba, R. Spears, P. Rosario, and S. Lamb. Reference Pathologists: J.C. LeRiche, British Columbia Cancer Agency, Vancouver, BC; A.F. Gazdar, Hamon Cancer Center, Dallas. Consulting Statisticians: A. Coldman, British Columbia Cancer Research Centre, Vancouver, BC; C. Dorrier, BRI International, Arlington, Va. Clinical Trial Monitors: D. Wisdahl, F. Catonio-Begley, and D. Middelaer, Xillix Technologies Corp.

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Autofluorescence Bronchoscopy – A Comparison of Two Systems (LIFE and D-Light)

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For editorial comment see p. 347

Key Words
Autofluorescence bronchoscopy · Bronchoscopy · Lung cancer

Abstract
Background: Autofluorescense (AF) bronchoscopy is an established method to detect dysplasia and carcinoma in situ (CIS). Several different systems are currently available. ***Objectives:*** This study aimed to directly compare the LIFE system (Xillix Technologies, Vancouver, Canada) and the D-light system (Storz, Tuttlingen, Germany). ***Methods:*** In a prospective study performed between May 1999 and October 2000, we examined patients with risk factors for lung cancer that underwent bronchoscopy with both (LIFE and D-light) systems in a crossover design. The findings were classified into normal, abnormal and suspicious lesions by independent investigators and then compared. ***Results:*** This study comprised 332 patients (220 males, 112 females, mean age 62.7 years, range 40–85); 1,117 biopsies were studied (mean biopsy rate 3.4/patient). In 817 biopsies, mucosal areas were classified as normal with respect to control biopsy specimens, 113 as abnormal and 187 as suspicious using AF bronchoscopy. The histological examination showed normal tissue in 850 cases, in 55 cases scarring or inflammation, in 62 meta- or dysplasias, in 11 carcinomas in situ and in 127 invasive tumors. In only 5 cases, classifications were found to be different between the two systems (2 normal, 2 dysplasias, 1 invasive tumor). The mean time for the LIFE system examination amounted to 11.7 min (range 6.2–19.5) and for the D-light system to 7.4 min (range 4.3–11.9). This difference was statistically significant ($p < 0.001$). ***Conclusion:*** Both systems yielded comparable results. The examination time was significantly shorter with the D-light system, which may be explained by the more comfortable handling and the direct switch between white light and AF imaging. Different trials using either methodology could be compared directly.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the United States and Europe [1]. In the 1999, roughly 170,000 cases have been newly diagnosed in the United States alone. When patients presenting with

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symptoms of the disease undergo a workup, usually they are found to have advanced disease and therefore limited treatment options. Five years after the initial diagnosis, only 15% of patients will be alive [1, 2]. These dismal numbers have lead to renewed interest in screening to detect lung cancer at earlier stages when a cure is still possible [3]. The recently introduced low-dose screening with chest CT appears promising and is currently investigated in clinical trials [4]. As a complementary method, autofluorescence (AF) bronchoscopy has been introduced into clinical practice to detect early cancerous lesions within the airways. 'Fluorescence' is a physical phenomenon, a form of luminescence or 'cold' light emission. It occurs when an object on which light of a certain wavelength is directed remits light of a longer wavelength. Its effect on body tissue has been recognized since the early 1900s ('Wood's light') [5]. In 1943, Herley [6] described fluorescence tissue reaction to ultraviolet light, noting even then that a tumor behaved differently than healthy tissue.

Previously, von Holstein et al. [7] and Lam et al. [8] have discovered that tumorous tissue could be distinguished from normal mucosa by a noticeable reduction in AF, the key step to the introduction of LIFE (lung imaging fluorescent endoscopy) bronchoscopy. When monochromal light of 442 nm is directed at mucosa, subepithelial fluorophores are stimulated to emit light of a longer wavelength. Reduced fluorescence is due to epithelial thickening, tumor hyperemia (hemoglobin absorbs virtually all the green light), and redox changes in the tumor matrix and reduced fluorophore concentration.

In 1998, Lam et al. [9] published the results of an initial trial performed with the LIFE system in 173 patients with suspected lung cancer in whom white light bronchoscopy was followed by AF. From a total of 700 biopsies, 142 were moderate-to-severe dysplasia, carcinoma in situ (CIS) or invasive cancer. Whereas 35 lesions were recognized by white light bronchoscopy, 91 were correctly identified using LIFE, corresponding to a relative sensitivity of 2.7.

In the recent past, newer AF systems have been introduced which are cheaper and more convenient in their handling, but their efficacy has not been compared to the established standard of LIFE bronchoscopy. Additionally, it is important to establish a comparison of different technologies in order to assess the ability to directly compare outcomes and findings in studies using different technologies. We report our experience with the two different AF systems.

Table 1. Indications for AF bronchoscopy

Strong smoker (>20 pack years)	and
Written informed consent	and
Postoperative care (treated bronchial carcinoma)	or
Radiological suspicion of carcinoma	or
Positive sputum cytology	

Patients and Methods

From May 1999 to October 2000, patients with risk factors for lung cancer were studied using the LIFE system (Xillix Technologies, Vancouver, Canada) and the D-light system (Storz, Tuttlingen, Germany) in a crossover design in the context of a prospective randomized study (table 1). Previous evaluation with fluorescence bronchoscopy was an exclusion criterion.

Changes that were considered abnormal during white light examination included areas presenting an increase in redness and a thickening or irregularity of the mucosa. The endoscopic assessment was performed according to Lam et al. [9]. During AF examination with both systems, abnormal areas corresponding to heterogeneous aspects of or homogenous defects in the mucosa were noted. Homogenous defects corresponded to areas characterized by a color that differed from the green appearance of the normal mucosa (i.e. black or red), whereas heterogeneous aspects corresponded to areas with patchy defects in the green normal appearance. At the end of the white light and AF examinations, biopsy specimens of all abnormal areas were obtained, as well as random biopsy specimens in areas that appeared normal during both white light and AF examination (control biopsy specimens).

All biopsy specimens were fixed in formalin and embedded in paraffin. They were classified by a single experienced lung pathologist who was unaware of the findings of bronchoscopy into the following categories: normal; reserve cell hyperplasia; metaplasia; dysplasia of mild, moderate, or severe grade; CIS, and invasive carcinoma. Additional histological changes including inflammation or scarring were by convention considered as 'other abnormalities'. Biopsies were not re-examined for study purposes.

The duration of the endoscopic examinations was noted. The operator could also control, under white light examination, areas that were considered abnormal during fluorescence examination. This control examination was only performed at the end of the fluorescence examination with the LIFE system because it required changing both the light source and the camera. With the D-light, the switch in the camera allowed immediate and repeated changes, and the number of control procedures was recorded. These controls were considered part of the AF examination.

LIFE System

LIFE employs a helium-cadmium laser light source, with two image intensifier CCD cameras and a color video monitor for imaging. The system is somewhat bulky and, due to the two cameras, it does not allow direct and immediate comparison of white light and AF imaging [10].

Table 2. Description of the patient cohort and indications for AF examination

Female	112
Male	220
Mean age, years	62.7
Postoperative follow-up	123
Suspicious chest X-ray	187
Positive sputum cytology	32

Table 3. Differences between LIFE and D-light findings in 5 cases

	Histology	LIFE	D-light
Case 1	normal	normal	abnormal
Case 2	normal	abnormal	normal
Case 3	dysplasia	normal	suspicious
Case 4	dysplasia	suspicious	abnormal
Case 5	CIS	normal	suspicious

Gold standard is the histologically associated finding obtained by endobronchial biopsy.

D-Light System

The D-light system uses a 300-watt xenon lamp, which is optimized for blue light transmission. In the AF mode, wavelengths from 380 to 460 nm are used for excitation. For detection, a filter integrated in the eyepiece of the bronchoscope blocks most of the excitation light. Light intensity and quality of image enable investigation under direct AF guidance. A single small camera enables the white light and fluorescence images, which can be directly observed under dimmed daylight [11].

Statistical Analysis

Randomization was performed using a computer protocol. Results are expressed as means and standard deviations. Wilcoxon's test was used to compare the two systems, and the criterion for statistical significance was $p < 0.05$.

Bronchoscopy

After informed consent was obtained, patients underwent standard bronchoscopy with flexible instruments (Olympus, Tokyo, Japan), followed by the respective AF endoscopy. Patients received topical anesthesia (1% lidocaine) and conscious sedation (fentanyl and midazolam) and in some cases general anesthesia if rigid examinations were preferred. Care was taken to inspect the mucosa without any suction trauma, and all biopsies were deferred to the end of the examination.

Results

The indications of the 332 patients (220 males, 112 females, mean age 62.7 years, range 40–85 years) included in the study are shown in table 2. Of 1,117 biopsies (mean 3.4/patient), 817 were obtained at endoscopic normal, 113 abnormal and 187 at suspicious sites. Histologically, 850 were classified as normal, scarring or inflammation was noted in 55, meta- or mild dysplasia in 62, severe dysplasia in 12, CIS/microinvasive carcinoma in 11 and invasive tumors in 127 specimens.

Differences in AF bronchoscopy were observed in 5 biopsies only ($p = 0.3$; table 3). Histologically, 2 were classified as normal (one each), 2 demonstrated severe dysplasia (detected by LIFE/missed by D-light) and 1 CIS (detected by D-light/missed by LIFE; fig. 1, 2). The rate of false-positive results obtained was 8% (37/187; fig. 1, 2).

The mean time (total for white light and AF) required for the examination with LIFE was 11.7 min (range 6.2–19.5 min) and with the D-light system 7.4 min (range 4.3–11.9 min). The difference was statistically significant ($p < 0.001$). No complications occurred.

Discussion

Several studies have reported that the addition of AF bronchoscopy to conventional WBL increases the detection rate of preneoplastic lesions and early lung cancers [9–12].

The primary aim of the current study was not to compare the sensitivity, specificity, and positive and negative predictive values of white light bronchoscopy with or without the addition of either the D-light system or LIFE AF, because this comparison would have required a much higher number of patients and lesions. The aim of this study was a direct comparison of the LIFE and D-light systems used to induce and observe endobronchial AF.

Both systems missed only one notable lesion (1 dysplasia missed by the D-light system and 1 CIS missed by LIFE), respectively. An important limitation of fluorescence bronchoscopy is the high rate of false-positive results: in a recent, large multicenter study, the positive predictive value of fluorescence bronchoscopy in the detection of lesions corresponding to moderate dysplasia or worse amounted only to 33% [9, 11]. This is probably explained, at least in part, by the fact that fluorescence examination provides qualitative and, therefore, subjective results. In the current study, AF examination was associated with the detection of a large number of abnor-

Fig. 1. Dysplasia (moderate): correct detection with the LIFE system but missed with the D-light system.

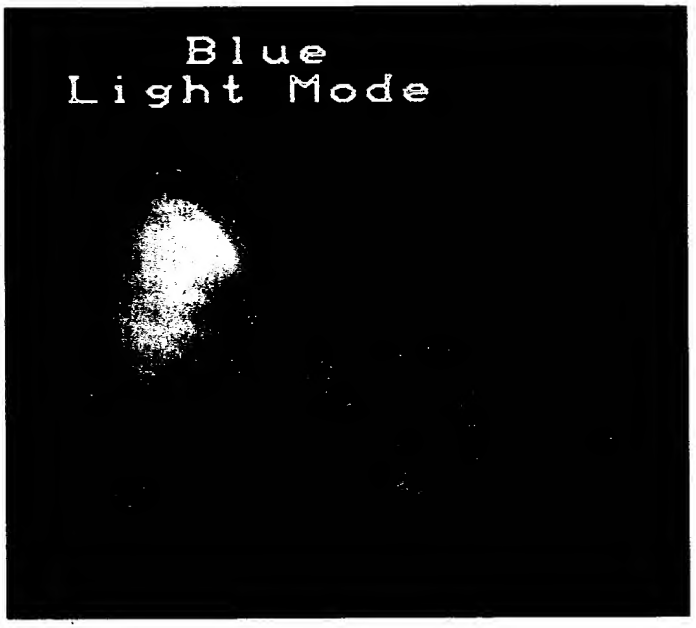

Fig. 2. CIS: correct detection with the D-light system but missed with the LIFE system.

mal areas that, in fact, corresponded to low-grade intraepithelial lesions, for example, but the false-positive rate was similar between systems.

The D-light system allowed for consistently shorter examination times. This is related to its easier handling and the ability to switch between white light and blue light with a button, rather than having to change light sources. In combination with a significantly cheaper price, the D-light system might thus be more attractive for many endoscopy units. In conclusion, the findings of this study indicate that the results of trials employing either technology can be directly compared.

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Effective detection of bronchial preinvasive lesions by a new autofluorescence imaging bronchovideoscope system

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Preinvasive lesion;
Magenta

Summary Autofluorescence bronchoscopy is an important tool for the early detection of preinvasive bronchial lesions. However, autofluorescence bronchoscopy has difficulty distinguishing between preinvasive lesions and other benign epithelial changes. A new autofluorescence imaging bronchovideoscope system (AFI) comprises three signals, including an autofluorescence (460–690 nm) on excitation blue light (395–445 nm) and two different bands of reflected light: G′ (550 nm) and R′ (610 nm). We hypothesized that color analyses of these three wave lengths would improve our ability to differentiate between inflammation and preinvasive lesions. In order to prove this hypothesis and to evaluate the efficacy of AFI for detecting preinvasive lesions, we conducted a prospective study. A total of 32 patients with suspected or known lung cancer were entered into this study. Conventional white light bronchovideoscopy (WLB) and light induced fluorescence endoscopy (LIFE) were performed prior to using AFI. WLB and LIFE detected 62 lesions, including lung cancers ($n=2$), squamous dysplasias ($n=30$), and bronchitis ($n=30$). By utilizing AFI, 24 dysplasias and 2 cancer lesions were magenta in color, while 25 bronchitis lesions were blue. The sensitivities of detecting dysplasia by LIFE and AFI were 96.7% and 80%, respectively. The specificity of AFI (83.3%) was significantly higher than that of LIFE (36.6%) ($p=0.0005$). We conclude that AFI appears to represent a significant

Abbreviations: AFI, autofluorescence imaging bronchovideoscope system; LIFE, laser-induced fluorescence endoscopy; WLB, white light bronchoscopy; CIS, carcinoma in situ; RGB, red/green/blue

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advance in distinguishing preinvasive and malignant lesions from bronchitis or hyperplasia under circumstances where LIFE would identify these all as abnormal lesions.

1. Introduction

Lung cancer is one of the most common causes of cancer-related deaths. Detection of tumors at an early stage followed by complete surgical resection of tumors is the only curative treatment approach currently available for the large majority of lung cancer patients. Laser-induced fluorescence endoscopy (LIFE) lung-system (Xillix Technologies Corp.; Richmond, BC, Canada) is a new modality that has facilitated early detection of preinvasive bronchial lesions [1–5]. However, LIFE has difficulty distinguishing between preinvasive lesions and other benign epithelial changes such as bronchitis, which frequently is present in patients whose sputum cytology is suspicious of malignancy or positive for malignancy [3,6]. Bronchoscopic findings have been divided into three classes: class I (normal), class II (inflammation and mild dysplasia), and class III (changes suggesting moderate or severe dysplasia, carcinoma in situ and/or invasive cancer) [5,7]. At present, it remains difficult to predict pathological diagnosis from the observed grade of autofluorescence, as studies have found that some lesions identified as class III turned out to be inflammatory lesions on biopsy [7,8]. The specificity of fluorescence bronchoscopy is reported to be rather low with up to one-third of the areas of abnormal fluorescence representing false positives [1,9]. Kennedy et al. note that this low specificity leads to unnecessary biopsies, resulting in greater economic cost and longer examination time [10]. Therefore, the ability to distinguish preinvasive lesions from bronchitis would be of great value to endoscopic technology. Recently, a new autofluorescence imaging bronchovideoscope (AFI) system (Olympus Optical Corporation, Tokyo, Japan) combining autofluorescence with two reflected light signals has been developed in an attempt to improve the diagnostic precision of fluorescence bronchoscopy. We have therefore conducted a prospective clinical study in order to evaluate whether, compared with LIFE, AFI can more precisely distinguish between preinvasive bronchial lesions and bronchitis.

2. Methods

2.1. Autofluorescence imaging videoscope system

AFI is a video-endoscopic system that displays a composite image integrating three signals—an autofluorescence signal plus reflected green (G′) and red (R′) light signals. The Storz D-light system has already combined autofluorescence and reflected light [11], but AFI has further developed this approach. Fig. 1 shows spectra of the three AFI signals and the absorption coefficient of HbO_2 (solid line). The excitation light (395–445 nm) produces an autofluorescence signal (490–700 nm). Green (G′)-light

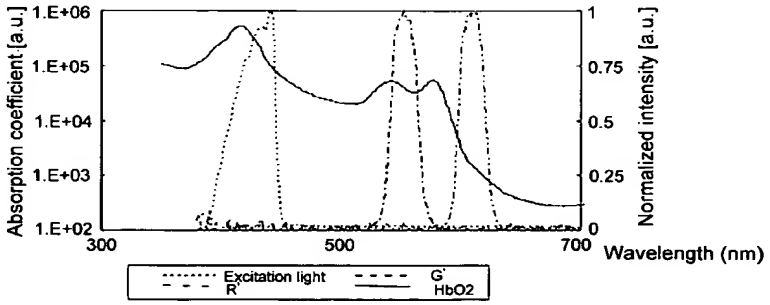

Fig. 1 Absorption coefficient of HbO_2 and spectrums of the illumination by AFI. The solid line is an absorption coefficient of HbO_2. The excitation light (395–445 nm), green (G′) light (550 nm) and red (R′) light (610 nm) are irradiated based on the RGB sequential videoscope system. The excitation light produces an autofluorescence signal (490–700 nm), while the G′ and R′ illumination lights generate G′ and R′ reflected signals, respectively.

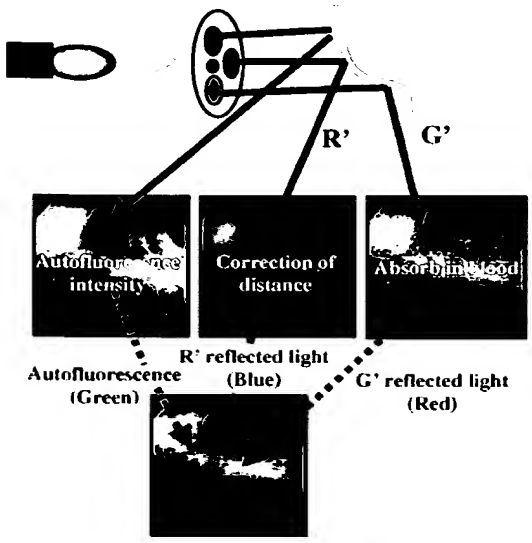

Fig. 2 Illustration of AFI system. The autofluorescence signal (490–700 nm) and the G′ and R′ reflected signals are integrated by the video processor. The composite image displayed on the monitor is constructed from displaying the autofluorescence as green, G′-reflected light as red, and R′-reflected light as blue.

(550 nm) and red (R′)-light (610 nm) generate G′ and R′ reflected signals, respectively. These three signals are irradiated based on the RGB sequential videoscope system, as illustrated in Fig. 2. These three signals are integrated by the video processor and a composite image is displayed on the monitor. The composite image is constructed by displaying the autofluorescence image as green, the G′-reflected light image as red and the R′-reflected light image as blue. Since hemoglobin absorbs a large volume of G′-light wavelength (550 nm) and little of the R′-light wavelength (610 nm), areas containing high levels of hemoglobin have decreased levels of autofluorescence (green) and/or G′-reflected light (red) but not R′-reflected light (blue). We previously reported that the detection of areas of increased vessel growth and complex networks of tortuous vessels in the bronchial mucosa may enable discrimination between bronchitis and dysplasia [12]. Therefore, we hypothesized that by comparison with ratios of above three-wave lengths which absorptions are influenced by hemoglobin concentration can be distinguished between bronchitis and dysplasia at the abnormal mucosa. AFI displays a light green image for normal epithelium and blue or magenta for an abnormal fluorescence, depending on the condition of abnormal epithelium.

2.2. Study population and bronchoscopic procedure

The Ethics Committees of the Graduate School of Medicine, Chiba University, approved all protocols and procedures. From October 2001 to May 2003, 32 patients comprising 25 patients with suspicious or malignant sputum cytology and 7 patients with known lung cancer were entered into this study. All participants provided written informed consent prior to the examination. Under local anesthesia, patients had their bronchial lesions evaluated in sequence by the following instruments: conventional white light bronchovideoscopy (WLB) using BF-240 (Olympus Optical Corporation, Tokyo, Japan), LIFE, and finally AFI. Biopsy specimens were obtained, fixed in 10% buffered formalin, and stained with hematoxylin and eosin. Two endoscopic specialists (MC and KS) evaluated all bronchoscopic findings. Two expert pulmonary pathologists at our hospital (KH and HO) determined and agreed on the final pathological diagnoses without having any information regarding the bronchoscopic findings. The relationship between bronchoscopic findings and pathological diagnoses was evaluated. A magenta image was classified as positive for malignant or preinvasive lesions, while a blue image was considered to represent inflammatory changes or bleeding. The pathological diagnoses were determined according to the third edition of "Histological typing of lung and pleural tumours" published by the World Health Organization International Histological Classification of Tumours in 1999 [13].

2.3. Color tone analysis

AFI images were stored in a BMP-image file format. The image color was resolved into the three primary colors of red, green, and blue. The data were subjected to a digamma operation to remove the gamma imposed on the signal at transmission. We created intensity histograms of the red, green, and blue components at the abnormal site and calculated the average intensity of each color for every 30 pixels using Adobe Photoshop 5.0 (Adobe Systems Incorporated, CA, USA). We used the intensity of the blue signal as the denominator and calculated the proportion of red intensity to blue intensity (red/blue) and the proportion of green intensity to blue intensity (green/blue) [8].

2.4. Statistical analysis

Data were analyzed using Stat View Version 5.0 (Statistical Analysis Systems; Cary, NC, USA). The Chi

square test was used to compare the diagnostic sensitivity and specificity, while Student's *t*-test was used to compare the continuous values.

3. Results

3.1. Representative case of bronchoscopic findings

Fig. 3A, B, and C show typical bronchoscopic findings of WLB, LIFE, and AFI for squamous cell carcinoma, squamous dysplasia, and bronchitis, respectively. Arrows indicate abnormal areas of bronchial epithelium. Fig. 3A shows a case of squamous cell carcinoma. Irregular mucosa and protruding nodules are identified at the bifurcation of the right middle and lower lobe bronchi by WLB, LIFE, and AFI. The AFI image clearly is magenta at the abnormal area. Fig. 3B shows a case of squamous dysplasia at the bifurcation of the left B3a and B3bc. Although WLB demonstrated normal bifurcation, LIFE demonstrated abnormal autofluorescence, and the AFI image was magenta. Fig. 3C shows a case of bronchitis affecting the bifurcation of the right B4 and 5. LIFE demonstrated abnormal autofluorescence and the AFI image was blue.

Fig. 3 Images of WLB, LIFE, and AFI in patients with squamous cell carcinoma (A), squamous dysplasia (B), and bronchitis (C). AFI depicted squamous cell carcinoma and squamous dysplasia as magenta while depicting bronchitis as blue. Arrows indicate abnormal mucosal changes.

3.2. Patient characteristics and diagnostic results

Study patients (30 males and 2 females) ranged in age from 35 to 78 years (mean 66.6 ± 9.8 years). Smoking history ranged from 0 to 153 pack-years (mean 50.9 ± 29). Only one study patient was a non-smoker and that this non-smoker was a female.

Sixty-two abnormal sites were recorded using WLB, LIFE, and AFI from which pathological diagnoses were obtained. The relationship between each bronchoscopic finding and the pathological diagnosis is shown in Table 1. Two squamous cell carcinomas (an early cancer and an invasive cancer) were recognized as abnormal by all three types of bronchoscopic examination. While WLB detected only 16 dysplastic lesions comprising 1 severe dysplasia, 13 moderate dysplasias, and 2 mild dysplasias, LIFE detected 29 dysplastic lesions comprising 2 severe dysplasias, 22 moderate dysplasias and 5 mild dysplasias. AFI revealed 24 dysplastic lesions comprising 2 severe dysplasias, 18 moderate dysplasias and 4 mild dysplasias.

Table 1 Relationships between the bronchoscopic findings of the three bronchoscopic technologies and the pathological diagnoses of the biopsy specimens

	WLB		LIFE		AFI	
	+	−	+	−	+	−
Bronchitis (30)	15	15	19	11	5	25
Mild dysplasia (6)	2	4	5	1	4	2
Moderate dysplasia (22)	13	9	22	0	18	4
Severe dysplasia (2)	1	1	2	0	2	0
Squamous cell carcinoma (2)	2	0	2	0	2	0

WLB: white light bronchoscope; LIFE: laser-induced fluorescence endoscopy lung system; AFI: autofluorescence imaging bronchovideoscope system.

Table 2 Diagnostic sensitivity and specificity of AFI and LIFE

	LIFE	AFI	*p*-Value
Sensitivity	96.7% (29/30)	80% (24/30)	0.1028
Specificity	36.6% (11/30)	83.3% (25/30)	0.0005

LIFE: laser-induced fluorescence endoscopy lung system; AFI: autofluorescence imaging bronchovideoscope system.

Since the two squamous cell carcinomas were detected by all three modalities, these samples were excluded from the analysis of diagnostic accuracy. The sensitivities of LIFE and AFI for detecting squamous dysplasia were 96.7% (29/30) and 80% (24/30), respectively ($p = 0.1028$) (Table 2). On the other hand, the specificity of AFI (83.3% 25/30) was significantly better than the specificity of LIFE (36.6, 11/30) ($p = 0.0005$).

3.3. Color tone analysis

We analyzed the color components of 60 lesions (excluding 2 cancer lesions) to evaluate the capability of AFI to distinguish dysplasia from bronchitis. Fig. 4A and B show representative pictures of abnormal findings identified by AFI. We calculated the three-color intensities of mucosa (Fig. 4 and Table 3) and compared them with the histology. The open squares of the dotted line show the abnormal areas. The three-color histograms show the distributions of red, blue, and green signal intensities. Fig. 4A shows a case of moderate dysplasia. The mean intensities of the red, green, and blue signals were 42.19 ± 12.19, 30.5 ± 2.56, and 21.31 ± 3.98 pixels, respectively. The red/blue, green/blue and red/green proportions of the mean intensities were 1.98 (42.19/21.31), 1.43 (30.5/21.31), and 1.38 (42.19/30.5), respectively (Table 3). Fig. 4B shows a case of bronchitis. The mean intensities of the red, green, and blue signals were 28.6 ± 3.53, 38.5 ± 1.7, and 24.7 ± 1.0 pixels, respectively. The red/blue, green/blue, and red/green proportions of the mean intensities were 1.16 (28.6/24.7), 1.56 (38.5/24.7), and 0.74 (28.6/38.5), respectively (Table 3). Comparison of the color tone ratio between squamous dysplasia and bronchitis indicated that red/blue and red/green ratios in squamous dysplasia were higher than those in bronchitis, while the green/blue ratios were equivalent. Fig. 5 shows the relationship between pathological diagnoses and color tone analyses. We plotted the proportions of red/blue and green/blue signal-intensities for the *Y*-axis and *X*-axis, respectively. Sixty biopsy samples were characterized as bronchitis ($n = 30$) or squamous dysplasia ($n = 30$). The average red/blue value for the 30 preinvasive lesions was 1.786 ± 0.371, which was significantly higher than the average red/blue value for bronchitis (1.243 ± 0.087, $p < 0.0001$). To determine the cut-off point of red/blue that had the best diagnos-

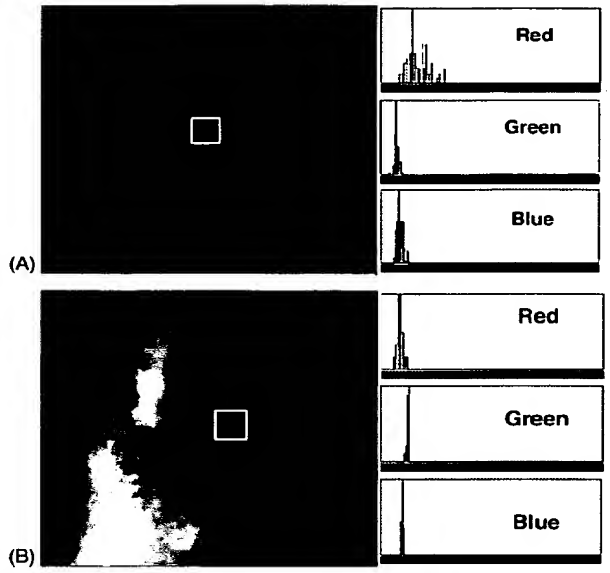

Fig. 4 Typical bronchoscopic findings of moderate dysplasia (A) and bronchitis (B) and their histograms using AFI. The *X*-axis indicates the intensity of each color (0–255 bits) and the *Y*-axis indicates the ratio of pixels (total 30 pixels) for each color pair (i.e. red, blue, and green).

Table 3 Representative three-color intensities and color ratios of red/blue and green/blue

	Red (G′ reflected)	Green (autofluorescence)	Blue (R′ reflected)	Red/blue	Green/blue
Bronchitis	28.6 ± 3.53	38.5 ± 1.7	24.7 ± 1.0	1.16	1.56
Bleeding	2.80 ± 1.96	4.91 ± 1.91	14.20 ± 1.57	0.34	0.19
Moderate dysplasia	42.19 ± 12.19	30.5 ± 2.56	21.31 ± 3.98	1.98	1.43
Severe dysplasia	16.73 ± 3.65	12.13 ± 1.26	7.17 ± 1.31	2.33	1.69
Cancer	33.38 ± 7.65	5.69 ± 0.59	17.96 ± 2.16	1.85	0.31

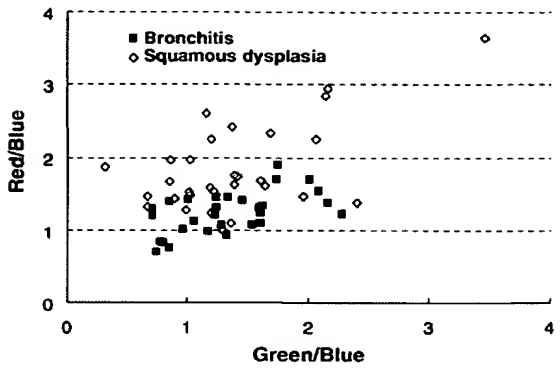

Fig. 5 Color tone analysis of AFI image. The color resolution correlated well with pathology and facilitated the distinction between squamous dysplasia and bronchitis.

tic specificity and accuracy, we established cut-off points at 0.05 intervals and calculated the sensitivity, specificity, and accuracy of each cut-off point. From this analysis, we determined that a red/blue cut-off point of 1.45 had the highest accuracy for diagnosing preinvasive lesions, with a sensitivity and specificity of 71.9% and 88.3%, respectively, yielding an accuracy of 77.4%.

4. Discussion

It has been reported that autofluorescence bronchoscopy is more useful for detecting preinvasive lesions than white light bronchoscopy [1]. The results of multi-center clinical trials and our own experience suggest that autofluorescence bronchoscopy increases the diagnostic accuracy for squamous dysplasia, CIS, and early hilar lung carcinoma when used simultaneously with conventional bronchoscopy [2,3,5,6,9,14,15]. Although several types of autofluorescence systems, such as the LIFE-Lung system, the SAFE-1000 (Asahi Optical Corp., Tokyo, Japan) [16], and the D-light system [11] have been developed, these systems have a low specificity for diagnosing preinvasive lesions [9,17]. Individuals at high risk for lung cancer also frequently suffer from bronchitis and hyperplasia due to cigarette smoking [5,6,7,18]. In a previous study of the usefulness of LIFE for detecting preinvasive lesions, we found that out of the 162 sites that LIFE identified as abnormal, the pathological diagnosis in 84 cases turned out to be hyperplasia, bronchitis or normal epithelium. This outcome suggests that specific visual diagnosis of preinvasive lesions by LIFE is still problematic [3]. LIFE images all areas of epithelial thickness and hypervascularity as mild red with suppressed green fluorescence [4]. To improve specificity, color differentiation between preinvasive lesions and bronchitis is necessary.

In the present study, we hypothesized that color tone analyses of three-wave lengths would improve our ability to distinguish between inflammation and preinvasive lesions. The AFI system incorporates autofluorescence (green), G′ reflected light (red) and R′ reflected light (blue). A loss of green autofluorescence is caused by increased thickness of the epithelial layer and the accumulation of certain substances in the tissue. Areas containing high concentrations of hemoglobin exhibit a decrease not only in green autofluorescence but also a decrease in G′ reflected light (red). R′ reflected light (blue) is less influenced by mucosal thickness and/or hemoglobin concentration. Squamous dysplasia is characterized by the presence of atypical cells and an increase in subepithelial microvessel density [19,20]. Since AFI preferentially images the condition of the bronchial epithelium rather than the condition of the submucosa, the decrease in intensity of green, which is influenced by both epithelial thickness and hemoglobin, is more pronounced than the decrease in the intensity of red, which is influenced by only hemoglobin. As a result, dysplasia produced a magenta color, a combination of red and blue, in the AFI image. Bronchitis is characterized by lymphocytic infiltration of the bronchial wall and associated blood vessel congestion [20]. Therefore, G′ reflected light (red) is more decreased than autofluorescence (green), and as a result, bronchitis produces a blue to green-blue color in the AFI image. Autofluorescence is the product of various factors, including thickness of epithelial layers, hemoglobin absorption, and the tissue concentrations of substances such as flavins, collagen, and nicotinamide adenine dinucleotide (NADH) [21]. Therefore, the green tone (autofluorescence) is variable. Since the blue tone (R′ reflected light) is more constant than both the green tone (autofluorescence) and the red tone (G′ reflected light), the green/blue (autofluorescence/R′ reflected light) ratio had a wide range for both bronchitis and squamous dysplasia.

Regarding the red/blue ratio, we were able to verify that the red/blue ratio for dysplasia was significantly higher than the red/blue ratio for bronchitis. When the cut-off point of the red/blue ratio was set at 1.45, 71.9% sensitivity, 88.3% specificity, and 77.4% accuracy were obtained. The results of the present study suggest that AFI may have the capability of objectively distinguishing between squamous dysplasia and bronchitis.

5. Conclusion

AFI could accurately and objectively distinguish preinvasive and malignant lesions from bronchitis through color tone analysis.

Acknowledgments

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ORIGINAL ARTICLE

Endoscopic video autofluorescence imaging may improve the detection of early neoplasia in patients with Barrett's esophagus (CME)

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Background: The aim of this study was to investigate the feasibility of detecting high-grade dysplasia (HGD) and early cancer (EC) in Barrett's esophagus (BE) with a prototype video autofluorescence endoscope.

Methods: Sixty patients with BE were evaluated with a prototype, high-resolution videoendoscope that has separate charge-coupled devices for white light endoscopy (WLE) and autofluorescence imaging (AFI). Nondysplastic BE appears green on AFI, whereas potentially neoplastic areas appear blue/violet. The BE was first screened with WLE for visible abnormalities and then was examined by AFI to detect additional lesions. Lesions that raised a suspicion of neoplasia and control areas that were normal on AFI were sampled for histopathologic assessment. Finally, random 4-quadrant biopsy specimens were obtained at 2-cm intervals.

Results: A diagnosis of HGD/EC was made in 22 patients; one patient had no visible abnormality, and 21 had endoscopically detectable areas with HGD/EC. In 6 of the latter 21 patients, the HGD/EC was detected with AFI alone; in another patient, HGD/EC was detected with AFI and random biopsies. In 14 patients, HGD/EC was detected with both WLE and AFI; in 3 of these 14 patients, additional lesions containing HGD/EC were detected by AFI alone.

Conclusions: The results of this study suggest that video AFI may improve the detection of HGD/EC in patients with BE. (Gastrointest Endosc 2005;61:679-85.)

Barrett's esophagus (BE) is widely recognized as a premalignant condition.[1] Many professional societies recommend regular endoscopic surveillance for all patients with BE to detect neoplastic changes at an early, curable stage.[2] Treatment is recommended for patients with high-grade dysplasia (HGD) or early cancer (EC) in BE. These lesions, however, are difficult to detect at standard endoscopy and often are small.[3] The current surveillance strategy is to take biopsy specimens from all endoscopically visible mucosal abnormalities, together with 4-quadrant biopsy specimens every 1 to 2 cm beginning at the proximal end of the gastric folds and then proximally to the squamocolumnar junction.[2] This biopsy protocol is labor intensive and is subject to sampling error, because only a small fraction of the Barrett's segment is sampled.

When tissues are exposed to light of short wavelengths (usually ultraviolet or blue light), some endogenous biologic substances (i.e., fluorophores) emit fluorescence light with a longer wavelength, i.e., autofluorescence (AF).[4] Point spectroscopy techniques with small probes inserted through the accessory channel of the endoscope have been used to demonstrate that non-neoplastic and neoplastic tissues in BE have different AF characteristics.[5,6] This led to the development of light-induced fluorescence endoscopy (LIFE), a technique in which the mucosa is excited with blue light and a real-time AF endoscopic image is produced, based on the ratio of red to green AF. Uncontrolled studies with LIFE prototypes suggest that LIFE may improve the detection of early neoplasia in BE.[7,8] In a randomized cross-over study, however, no increase in the rate of detection of HGD or EC was found in BE when using LIFE (see Kara et al[9] in this issue). There are two possible explanations for these results. First, in the currently available prototypes,[9,10] the algorithm used to construct the AF image may not be optimal for BE; it only accounts for the ratio of red to green AF and does not incorporate information from reflected light. Second, the LIFE systems studied included fiberoptic endoscopes,[9,10] which provide a poor white-light image compared with currently available, high-quality videoendoscopes.

See CME section; p. 729.

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A newly developed prototype video AF endoscopy system may address the most important limitations of earlier prototypes. In this system, a high-resolution videoendoscope is used to produce high-quality white-light images. Moreover, a different algorithm is used to construct an AF image. The aim of the present study was to investigate the feasibility of detecting HGD and EC in patients with BE when using this new prototype.

Capsule Summary

What is already known on this topic

- Autofluorescence imaging (AFI) may detect dysplasia and neoplasia in Barrett's esophagus.
- When AFI is used, nondysplastic Barrett's esophagus appears green, whereas dysplastic or neoplastic lesions appear blue or violet.

What this study adds to our knowledge

- In a pilot study using video autofluorescence imaging, AFI detected more dysplastic and neoplastic changes in Barrett's esophagus than did conventional endoscopy with biopsies.

PATIENTS AND METHODS

Patients

Patients were included if they were known to have BE and were scheduled for EGD for one of the following indications: (1) regular surveillance, (2) evaluation of newly diagnosed HGD/EC, or (3) follow-up after endoscopic treatment of HGD/EC in BE. Patients with endoscopic evidence of erosive reflux esophagitis were excluded. The study protocol was approved by the medical ethics committee of our institution, and informed consent for participation in the study was obtained from all patients before the procedure.

Imaging system

The prototype AF endoscopy system used in this study (Olympus Optical Co, Ltd, Tokyo, Japan) includes a high-resolution videoendoscope and an AF imaging (AFI) modality. It has a xenon light source (XCLV-260HP; Olympus), with a rotary red/green/blue band-pass filter. With this light source, the mucosa is sequentially illuminated with red, green, and blue light at a frequency of 20 cycles per second. The high-resolution videoendoscope in this system has two separate monochromatic charge-coupled devices (CCD); one for white-light endoscopy (WLE), and one for AFI. In the WLE mode, the reflected red, green, and blue light is detected by the standard CCD and is converted to an electronic signal that is passed to the videoprocessor, which is synchronized with the rotary filter. The processor electronically overlays the red, green, and blue signals to produce a high-quality white-light image. In the AFI mode, blue spectrum light ($\lambda_{395\text{-}475\ nm}$) is delivered for excitation of AF, together with light in the green ($\lambda_{540\text{-}560\ nm}$) and the red ($\lambda_{600\text{-}620\ nm}$) spectra. The AFI-CCD has a barrier filter that allows detection of all light with a wavelength between 490 and 625 nm, thereby eliminating the blue excitation light. The sequentially detected images from AF, green reflectance, and red reflectance are integrated by the image processor into one AF image. The AF image, therefore, has 3 spectral components: (1) total AF in response to blue light excitation, (2) green reflectance light, and (3) red reflectance light. WLE and AFI can be alternated by means of a switch located conveniently on the endoscope. During endoscopy in the AFI mode, normal squamous mucosa and non-dysplastic BE appears green, whereas that which could potentially contain HGD/EC within the Barrett's segment has a bluish/purple AF color (Figs. 1 and 2). Small squamous islets within the Barrett's segment have a pinkish color.

Endoscopic procedure

Patients underwent upper endoscopy while under conscious sedation (intravenously administered midazolam, 5-10 mg). Per routine, the BE was first inspected with WLE. The length of the BE was measured as the distance from the proximal edge of the gastric folds to the squamocolumnar junction. The esophagus was further inspected for signs of reflux esophagitis and any visible mucosal abnormalities that raised a suspicion of HGD/EC. The location (distance from upper incisor teeth and endoscopic quadrant), as well as the macroscopic classification[11] of each lesion were recorded. Subsequently, the same inspection procedure was repeated after switching to the AFI mode. All areas within the BE with a blue/purple AFI color were regarded as suspicious for HGD/EC.

For every lesion, it was noted whether it was detected by WLE, AFI, or both. Still images were made of all lesions with both imaging modalities. Then, two to 4 biopsy specimens were obtained from each abnormality. In addition, control still images and corresponding biopsy specimens were obtained from areas that appeared green by AFI. Finally, 4-quadrant biopsy specimens were obtained according to the so-called Seattle protocol.[12] Biopsy specimens were taken beginning distally: first from lesions detected by AFI, followed by WLE-visible lesions, and finally random 4-quadrant biopsy specimens. If blood oozing from earlier biopsy sites impaired visualization of other lesions, the mucosal surface was irrigated with water until the target lesions were visible. All procedures were performed by two endoscopists experienced in the endoscopic detection and treatment of Barrett's neoplasia (P.F., J.B.). The endoscopists were aware of the clinical history of each patient, including the indication for endoscopy.

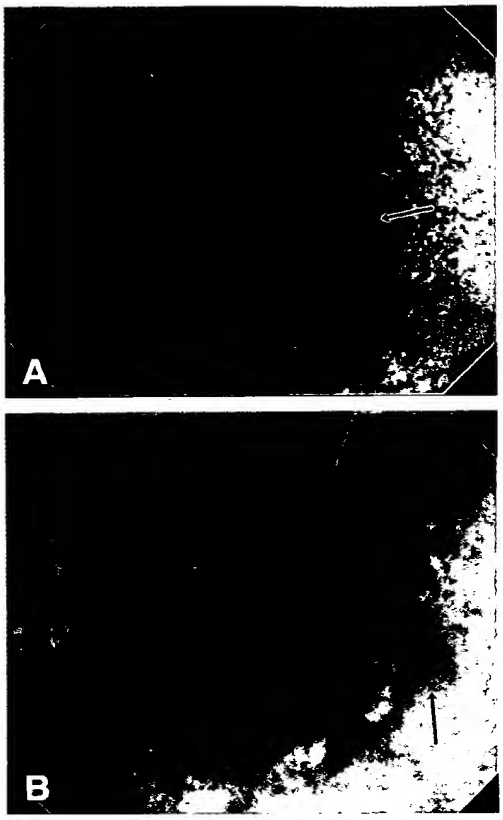

Figure 1. A, Retroverted white-light endoscopic view of Barrett's esophagus, showing focal lesion *(arrow)* with HGD. **B,** Corresponding retroverted AF image, showing focal lesion *(arrow)* with HGD. This lesion was first detected by AFI and only retrospectively seen with WLE (it was classified as "detected with AFI").

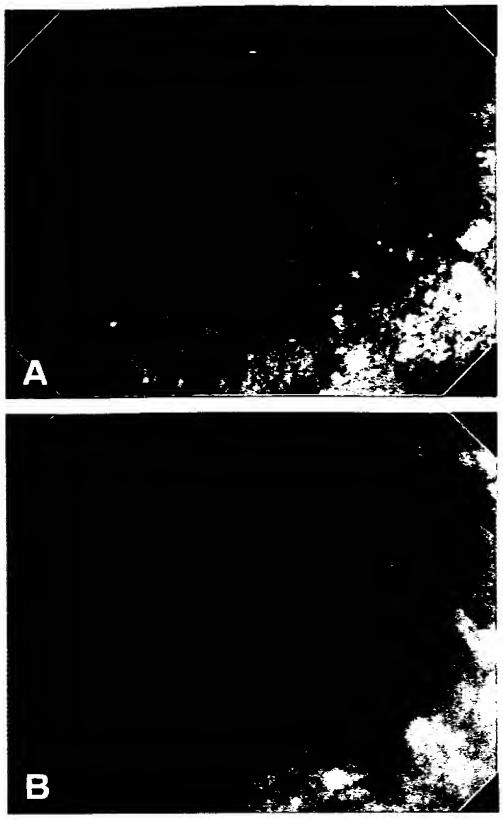

Figure 2. A, White-light endoscopic view of the distal esophagus in a patient with Barrett's esophagus. **B,** Corresponding AF image, showing blue/purple color *(right side)*, which raised a suspicion of HGD/EC, and greenish color *(left side)*. Biopsy specimens from the blue/purple area revealed EC; those from the greenish area revealed no dysplasia. This lesion was first detected with AFI and only retrospectively by WLE (it was classified as "detected with AFI").

Histopathologic assessment

All biopsy specimens were processed by using standard procedures and were systematically evaluated by an expert GI pathologist (F.t.K.) who was unaware to the results of the endoscopic imaging. A standardized form was used to assess each biopsy specimen for the presence, the grade, and the percentage of dysplasia; the presence and the degree of acute and chronic inflammation; and the presence of reactive epithelial changes. Dysplasia was classified into the following: no dysplasia, indefinite for dysplasia, low-grade dysplasia, HGD, and carcinoma, according to the microscopic classification of GI neoplasia.[13] The degree of dysplasia was semiquantitatively scored as focal (<10%), moderate (10%-50%), or diffuse (>50%). Acute inflammation was diagnosed if polymorphonuclear leukocytes were present. Chronic inflammation was determined by the presence of lymphocytic and plasmacellular infiltrates. Acute and chronic inflammation was semiquantitatively classified according to the density of the inflammatory infiltrate: less than 10 cells per high-power field was regarded as mild; dense clusters of cells were scored as severe. Reactive epithelial changes were defined as the presence of cell nuclear polymorphism without an increase in the nuclear/cytoplasmic ratio, along with a background of a stromal (and sometimes intraepithelial) inflammatory infiltrate. Acute and chronic inflammation and reactive epithelial changes were assessed to determine whether the finding of false-positive lesions might be explained by these processes.

Outcome parameters

Outcome parameters were the following: (1) the value of AFI in terms of the additional number of patients and the additional number of lesions with HGD/EC diagnosed after WLE; (2) the positive predictive value (PPV) and the negative predictive value (NPV) of AFI; and (3) the presence and the degree of acute inflammation, chronic inflammation, and reactive epithelial changes in AFI-guided biopsies (both from areas suspected to contain HGD/EC and from those without this suspicion). The PPV was calculated as the percentage of true positive lesions (i.e., with histologic confirmation of HGD/EC) among the

TABLE 1. The additional value of AFI in detecting HGD and EC in a selected group of 60 patients*

Category	Value
Per patient	1. 6/60 (10%) patients diagnosed with HGD/EC
	2. Increased the detection rate of HGD/EC from 23% to 33%
	3. 11 occult lesions with HGD/EC detected in 6 patients (3 patients with diffuse and 3 with multifocal HGD/EC)
Per lesion	100% increase in the total number of true positive targeted lesions (from 20 to 40 lesions)

AFI, Autofluorescence imaging; *HGD*, high-grade dysplasia; *EC*, early cancer.
*Of these 60 patients, 22 (37%) were diagnosed with HGD/EC.

total number of abnormalities detected with AFI. The NPV was calculated as the percentage of true negative samples (i.e., with histologic confirmation of the absence of HGD/EC) among the total number of control samples taken from areas with a green color on AFI.

Statistical analysis

Continuous variables with a Gaussian distribution were described as the mean and standard deviation, whereas those with a non-Gaussian distribution were described as the median and the interquartile range (IQR). The chi-square test (Fisher exact test when appropriate) was used to compare the degree of acute and chronic inflammation and the presence of reactive epithelial changes between false-positive lesions and control lesions. All statistic analyses were performed by using a statistical software package (Statistical Package for the Social Sciences 11.5; SPSS Inc, Chicago, Ill).

RESULTS

Patients

Sixty patients with BE (54 men, 6 women; mean age 65 [11] years) were included in this study. The median length of BE was 3 cm (IQR 2-7 cm). The indication for endoscopy was the following: regular surveillance with no history of HGD/EC (19 patients), evaluation of recently diagnosed HGD/EC (23 patients), and follow-up after endoscopic treatment for HGD/EC (18 patients).

Per patient analysis (additional value of AFI)

Of the 60 patients included, 22 were found to have HGD/EC (37%) by the study endoscopy. In 14 of these 22 patients, a total of 20 lesions with HGD/EC were detected with WLE; all of these lesions were identified as potentially HGD/EC by AFI. In 6 other patients, there was no abnormality detected with WLE, and no HGD/EC was found in random biopsy specimens; in these patients, the diagnosis of HGD/EC was made only by detection of 8 lesions with AFI. In another patient, there was one lesion with HGD/EC that was detected by AFI, although one of the random biopsy specimens also revealed HGD. Finally, one patient with HGD/EC had no visible abnormality detected by WLE or AFI, but one of the random biopsy specimens revealed HGD. When the endoscopic images from this patient were examined retrospectively, an area was noted on AFI that had a color that raised a suspicion of HGD/EC, but this observation was not made during endoscopy. Subsequent review of the digital video recordings confirmed that the single biopsy specimen with HGD was indeed taken from this misinterpreted area.

In total, there were 20 additional HGD/EC lesions detected by AFI alone. Eleven of these areas were detected in 6 of the 14 patients with lesions detected with both imaging modes. In 3 of these patients, however, there was diffuse HGD in the entire Barrett's segment, because random biopsy specimens also revealed HGD/EC. In the other 3, random biopsy specimens did not demonstrate HGD/EC.

To summarize, the additional value of AFI was the following: (1) the diagnosis of HGD/EC in 6 of 60 patients examined (10%), thereby increasing the detection rate of HGD/EC from 23% to 33%; (2) an increase in the total number of detected HGD/EC lesions from 20 to 40 (100%); and (3) the detection of occult multifocal HGD/EC in 3 patients, who had other lesions with HGD/EC detected with WLE (Table 1).

TABLE 2. Histopathologic evaluation of all AFI-guided biopsy specimens

AFI	NDBE	IND	LGD	HGD/EC	Total
Suspicious	28	5	8	40	81
Not suspicious	24	4	3	4	35

AFI, Autofluorescence imaging; *NDBE*, nondysplastic Barrett's esophagus; *IND*, indefinite for dysplasia; *LGD*, low-grade dysplasia; *HGD*, high-grade dysplasia; *EC*, early cancer.

Per lesion analysis

PPV and NPV of AFI. The histopathologic diagnoses for all AFI-guided biopsy specimens are shown in Table 2. In total, 81 areas were detected with AFI that raised a suspicion of HGD/EC. In 40 of these areas, HGD/EC was found in the corresponding biopsy specimens; the PPV of AFI, therefore, was 49%. Thirty-five biopsy specimens were taken from areas with a green AFI color (control biopsy specimens); 4 of these contained HGD upon histopathologic examination (Table 3). The NPV of AFI,

therefore, was 89% (31/35). The 4 false-negative samples were from 3 different patients, all of whom had a visible lesion detected with both WLE and AFI, and in whom all other biopsy specimens revealed HGD, thereby indicating diffuse HGD in the rest of the Barrett's segment. In addition, the HGD in these 4 biopsy specimens was focal (< 10%). In contrast, the percentage of dysplasia in true-positive AFI-guided lesions was diffuse (>50%) in 75% (15/20), moderate (>10% < 50%) in 5% (1/20), and focal in 20%.

Inflammation and reactive changes. In total, there were 41 false-positive lesions detected with AFI. The histopathology of these lesions was compared with the 31 true-negative samples for the following: (1) degree of acute inflammation, (2) degree of chronic inflammation, and (3) presence of reactive epithelial changes. Of these factors, the rate of moderate to severe acute inflammation was higher in biopsy specimens from false-positive lesions than in control biopsy specimens ($p = 0.009$, Fisher exact test) (Table 3).

TABLE 3. The degree of acute and chronic inflammation and the presence of reactive epithelial changes in AFI-guided biopsies: comparison between false-positive lesions and true negative areas*

Aspect	Severity	False-positive lesions	Control areas	p Value
Acute inflammation	Moderate to severe	8	0	0.009†
	Absent to mild	33	31	
Chronic inflammation	Moderate to severe	18	16	0.52‡
	Absent to mild	23	15	
Reactive epithelial changes	Present	35	28	0.72†
	Not present	6	3	

*The degree of acute inflammation showed a statistically significant difference between the two groups.
†Fisher exact test.
‡Pearson's chi-square test.

DISCUSSION

This study investigated the feasibility of detecting HGD and EC in BE by using a prototype of a novel endoscopic imaging technique, AFI. This prototype has some advantages compared with earlier prototypes of AF endoscopic imaging systems. First, the white-light image is of high-quality and is clearly superior to that provided by the fiberoptic endoscopes used in earlier prototype systems. Second, the AF image has 3 different components: total detected AF, green reflectance, and red reflectance. Incorporation of the red reflectance in the algorithm may correct for geometric (e.g., distance) variations that may cause a shadowing effect, which can be misinterpreted as an abnormality.[14] In addition, false-positive abnormalities in the image may be caused by inflammation, which is accompanied by a local increase in Hb content. Inclusion of the green reflectance image, at a wavelength band where the absorption by Hb is high, may reduce the confounding effects of inflammation.[15]

During endoscopy in the AFI mode, normal squamous mucosa and nondysplastic BE appear green, whereas areas within the Barrett's segment that may contain HGD/EC have a bluish/purple color (Figs. 1 and 2). Compared with previous prototypes, the AFI image provided by the current prototype seems to have an excellent contrast between nondysplastic and normal regions, and those suspected to contain HGD/EC. With previous prototypes, areas of suspected HGD/EC had a brick-red color against the already reddish background of the nondysplastic BE.[7-10]

Among the 22 patients found to have HGD/EC in this study, this diagnosis was made in 14 patients with both WLE and AFI. In 6 patients, HGD/EC was detected only with AFI-guided biopsy specimens, whereas other per-protocol random biopsy specimens were negative. Thus, AFI increased the detection rate of HGD/EC from 23% (14/60) to 33% (20/60). In addition, in 6 of the 14 patients with lesions visible with WLE and AFI, a total of 11 other foci of HGD/EC were detected by AFI alone. In 3 of these patients, the random biopsy specimens also revealed HGD; but, in the other 3, these biopsy specimens were negative, while AFI contributed to the discovery of multiple foci with HGD/EC. Therefore, AFI detected a substantial number of additional areas with HGD/EC that were not evident by WLE or detected in the random biopsy specimens.

Although the results of the current study are promising, the study has some limitations. First, endoscopic inspection with WLE and AFI was performed sequentially during the same procedure by the same endoscopist. Thus, the endoscopist was not blinded to lesions detected by WLE while examining the esophagus with AFI. Hence, the possibility of information bias in favor of AFI cannot be excluded. Second, each patient was examined only once, with biopsy specimens taken from all detected lesions, followed by procurement of random biopsy specimens according to the Seattle protocol.[12] Thus, there still is a chance of sampling error and that areas with HGD/EC were missed by WLE, AFI, and random biopsies. In a previous cross-over study, examination with a standard videoendoscopy and Seattle biopsy protocol missed about 20% of HGD/EC (see Kara et al[9] in this issue). The percentage of missed HGD/EC in the present study is

expected to be lower because of the better white-light image and the additional value of AFI. Nevertheless, it cannot be excluded that some areas with HGD/EC could have been missed. Furthermore, our institution is a tertiary referral center for the evaluation and the endoscopic treatment of patients with HGD and EC in BE. This referral bias, and the fact that the endoscopists were aware of the clinical history of the patients, including any previously detected dysplasia, may explain the high rate of HGD/EC in the present study. Nonetheless, this setting and this type of patient population are well suited to a feasibility study of the value of a new endoscopic imaging technique.

Although AFI appeared to add significant value in the present study, it was associated with a relatively high number of false-positive lesions; the PPV of finding an AFI-positive lesion was 49%. Note that this study was performed in a selected group of patients with BE in which 37% had HGD/EC. In patients with a lower risk for neoplasia (i.e., the standard surveillance population), it is likely that the PPV may be lower than the observed 49%. Several explanations are possible for some of the false-positive observations in the present study. First, a small rim of the BE immediately adjacent to the squamocolumnar junction often has a bluish color on AFI, which may lead to an increase in the false-positive rate of diagnosis. Second, areas with increased vascularity as seen by WLE exhibit a bluish color on AFI. Third, the gastric mucosa also appears blue/purple with AFI. Therefore, in a nondysplastic Barrett's segment, the proximal end of the gastric folds is characterized by a shift from a blue/purple to a green color. This may make it difficult to detect areas with HGD/EC that are extremely close to the proximal end of the gastric folds.

To investigate the causes of the high false-positive rate, all biopsy specimens were evaluated for the presence and the degree of acute and chronic inflammation and reactive epithelial changes. It appeared that biopsy specimens from false-positive areas had a significantly higher rate of acute inflammation compared with control biopsy specimens. Distinguishing areas with a severe acute inflammatory infiltrate from HGD/EC, therefore, may be difficult with the current settings for this prototype imaging system. In the endoscopic surveillance of patients with BE, however, the sensitivity and NPV for HGD/EC may be more important than the PPV of the technique, because the current standard is random 4-quadrant biopsies in the absence of visible lesions. The NPV of AFI was 89%. Among a total of 35 negative areas, 4 contained HGD upon histopathologic examination. These biopsy specimens were taken from 3 different patients who had a lesion detected by WLE and AFI (proven to be EC) and diffuse HGD in the entire Barrett's segment, because all random biopsy specimens revealed HGD. This means that these patients would not have been misclassified as having no HGD/EC.

In conclusion, the prototype endoscopic video AFI system evaluated in this study will increase the relative number and yield of targeted biopsy specimens. However, it will not eliminate the need for random biopsies unless randomized controlled studies demonstrate the contrary. This is illustrated by two patients in the current study: the patient in whom a focal area with HGD was detected with AFI, but one random biopsy specimen also revealed HGD; the patient with no endoscopically detectable abnormality on WLE and AFI, whereas retrospective examination of the digital images and video recordings from this patient demonstrated that the single biopsy specimen with HGD was obtained from an area just above the gastric folds that was abnormal on AFI. The latter patient was one of the first examined with this technique, which may suggest a learning curve effect.

This is the first study of the feasibility of video AFI in patients with BE. The results are promising. However, the data pertain to an uncontrolled feasibility study conducted in a high-risk population. Randomized cross-over studies, therefore, are required to investigate the true additional value of AFI over standard videoendoscopy. The technique also needs to be evaluated in low-risk patients with Barrett's esophagus to further assess its clinical relevance.

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REFERENCE 5

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New Incoherent Autofluorescence/Fluorescence System for Early Detection of Lung Cancer

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A new autofluorescence (AF) system for bronchoscopy that operates as compact as a conventional white light bronchoscopy system is described. The system is also capable of white light illumination and excitation of aminolevulinic acid (ALA) induced fluorescence. Changing between white light and (auto-) fluorescence mode is easy and always possible. Broad band excitation with blue light (AF: 380–460 nm; ALA 380–440 nm) delivers high intensity illumination at the distal end of the bronchoscope (AF typically 50 mW). A special optical observation technique makes the AF directly visible to the eye instead of indirect techniques used in other AF systems. A compact (160 g) and sensitive (typically 0.2 lux) camera can be used for documentation.

Keywords: ALA, Autofluorescence, Bronchoscopy, D-Light AF, Early detection, Lung cancer

INTRODUCTION

Perception depends upon the detection technique. What we see is the result of the brilliant possibilities of the human eye and the human brain. Vision enables us to orient and survive in our natural surroundings. However, only a fraction of all optical information is processed in the natural visual chain.

Going beyond normal vision is not necessary for living but can reveal a lot more information about the object being observed. Cleverly capturing this additional information opens the door to new analytical and diagnostic techniques. One of these new techniques is to reveal information hidden inside tissue to discriminate between suspect tumor tissue and normal tissue.

Basics of Fluorescence Imaging

Common visual pictures correlate the remission spectrum of an object with their physical properties: e.g. color, shape, texture, orientation and motion. The natural objective is to recognize known structures. Incident light $L(\lambda, x)$ as a function of wavelength λ and space x, which usually is, "white" sunlight, illuminates the scenery and the objects modulate the incident light due to different absorption and scattering characteristics $A(\lambda, x)$. The

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remitted scattered light $I_{A,S}(\lambda, x)$ has the same wavelength λ_r as the incident light λ_i.

$$I_{A,S}(\lambda, x) = A(\lambda, x) \cdot L(\lambda, x) \quad \lambda_r = \lambda_i = \lambda.$$

For example, blood absorbs light in the blue and green part and remits the remaining spectrum; the color of blood is described as red. This type of modulation is the dominant process which gives us orientation in our natural surrounding and in day-to-day life.

Fluorescence in contrast is an additional effect that contributes to remission and changes the wavelength of incident light. Fluorescence $F(\lambda_{Fl}; \lambda_i; x)$ correlates the wavelength of the incident light λ_i and the fluorescence light λ_{Fl} and therefore provides additional information. Generally speaking the fluorescent light I_{Fl} is red shifted.

$$I_{Fl}(\lambda_{Fl}, x) = \int F(\lambda_{Fl}; \lambda_i; x) \cdot L(\lambda_i; x)\, d\lambda_i \quad \lambda_{Fl} > \lambda_i.$$

Thus the overall intensity $I(\lambda, x)$ becomes

$$I(\lambda, x) = I_{A,S}(\lambda, x) + I_{Fl}(\lambda_{Fl}, x)$$

and is generally dominated by absorption and scattering effects, because fluorescence is weak.

By employing a simple trick we can make fluorescence visible, i.e. we have to eliminate the dominating scattering contribution to realize the fluorescence. This is possible due to the spectral separation of incident and fluorescent light. By blocking the wavelength of the incident light in the observation pathway we eliminate disturbing scattering light and reveal the fluorescence.

Fluorescence originates in chromophores which can be intrinsic (autofluorescence, AF) or added to the tumor tissue (xenofluorescence).

Biophysical Effects of Autofluorescence

To evoke AF in the visible range, special molecules or chromophores are required. Various proteins are known to display fluorescence when excited in the near UV or with blue light. These different

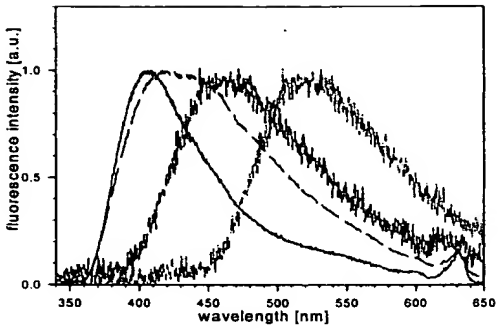

FIGURE 1 Normalized fluorescence spectra of various tissue chromophores in aqueous solution excited at 308 nm (from left to right: Collagen I, Collagen II, NADH, $FADH_2$).

chromophores are embedded in complex matrices and provide different micro environments to the chromophores. Excitation and fluorescence spectra of the dyes involved are typical broad band spectra. This effect can be used in the clinical application of tissue intrinsic (auto-) fluorescence.

Figure 1 displays several molecules which are expected to play an active role in AF when excited with a XeCl-Laser at 308 nm [1]. This representation is chosen for a more complete display of the spectra. Excitation with the KARL STORZ D (Diagnosis)-Light AF cuts off the short-wavelength part of the spectra.

The penetration depth of light is wavelength dependant and increases with wavelength in the visible range. Blue light penetration depth is limited to a fraction of a millimeter but is significantly deeper than UV light penetration depth. Therefore only the very top layers of tissue can be involved in optical effects. Fluorescence is suspected to originate in the submucosal layer.

The modulation responsible for contrasting early stage tumors is not revealed in detail. One effect is modified metabolic activity in tumor cells which effects also fluorescence characteristics. Another effect is tissue optical based and comes from the epithelium which absorbs light depending on the thickness of the layer (Fig. 2) according to Lambert–Beer's law. Malignant alterations display thickened epithelium and therefore appear darker under AF detection. Additionally, only a smaller

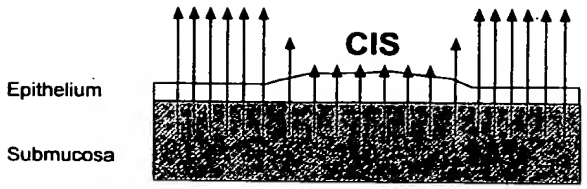

FIGURE 2 Tissue optical effect revealing early tumor stages, e.g. carcinoma *in situ* (CIS). Excitation at 380–460 nm; detection at 470–800 nm (schematical drawing).

amount of submucosal tissue can be illuminated due to limited penetration depth.

Biophysical Effects of ALA-induced Fluorescence

Inhalation of the endogeneous substance 5-amino-levulinic acid (ALA) leads to a tumor sensitive fluorescence marking. The heme metabolic pathway is made use of and transforms ALA into proto-porhyrin IX (PPIX) which is accumulated in tumor cells. PPIX shows an absorption maximum at 410 nm and fluorescence with broad peaks at 635 and 705 nm. Details are available in this volume (Huber *et al.*) and elsewhere [2].

The method of ALA-induced detection of early tumor stages is successfully applied with D-Light excitation for detection of superficial bladder cancer [3], malignant glioma [4] and in various other fields.

MATERIALS AND METHODS

History The incoherent light system used to induce fluorescence is the new KARL STORZ D-Light AF system (KARL STORZ, Tuttlingen, Germany) which is based on a technology developed for early detection of superficial bladder cancer [3]. This incoherent diagnostic approach for ALA-induced fluorescence was found to be clinically more convenient than a krypton-ion laser which originally was used for illumination [5]. Rapid technological innovation has created a powerful bronchoscopy system capable of (auto-) fluorescence detection. No contrast agent is required with the autofluorescence technology.

D-Light AF System

The D-Light AF system is handy, weighs less than 10 kg and is capable of three different illumination modes: (1). Conventional white light mode; (2) AF mode; (3) ALA-induced fluorescence mode which is described elsewhere [2]. The system is based on a 300-W Xenon lamp with special optics to focus high intensities of light into a liquid light guide, which is optimized for blue light transmission. The modes can easily be switched any time by a footswitch. In the AF mode output wavelength is between 380 and 460 nm; in the ALA mode between 380 and 440 nm. Blue light output power for AF at the distal end of a KARL STORZ broncho-fiberscope is typically 50 mW.

Observation

Both rigid telescopes and flexible fiberscopes are compatible with the KARL STORZ D-Light AF system. Critical to this method of tumor detection is the use of the correct observation technique as mentioned above which is realized in these special endoscopes. The specially designed endoscopes contain a filter wheel with three different positions for white light mode (a), for AF mode (b) and optionally for ALA-induced fluorescence mode (c). Position (a) does not contain any filter to allow standard white light bronchoscopy, whereas positions (b) and (c) contain observation filters that block the incident light properly for AF or ALA mode, respectively. For optimal contrast, specificity, illumination and plasticity a small amount of the blue excitation light bypasses the detection filters. With this trick, observation of biopsy forceps or other instruments is ensured even in (auto-) fluorescence mode.

The complete procedure can be performed entirely with D-Light AF and the special bronchoscope, and allows detection with the naked eye which is known for its high dynamic range. The technique used is direct imaging instead of indirect as used in other approaches [6].

The rigid telescopes used are Hopkins bronchoscopic telescopes with 5.5 mm diameter and

different directions of view (0° and 30°). They are optimized for fluorescence observation as described above. The 0° telescope provides extremely high luminous intensity so that white light conditions can almost be matched in the AF mode to allow perfect imaging and observation.

The fiberscopes were remodeled, improved and adapted for (auto-) fluorescence, with both a 5.0 mm (11001 BI) and 6.4 mm version (11004 BI) available. The distal tip has a upward range of 180° movement and a downward range of 100°/80°, respectively. Instrument channels are 2.3/2.8 mm, respectively. Optical quality is brilliant in both (auto-) fluorescence and white light mode.

Documentation

To allow proper documentation special endocameras are available (Telecam SL PDD, Tricam SL PDD, both KARL STORZ, Tuttlingen, Germany). These CCD-cameras have a optimized optical system and allow additional on-chip integration for the fluorescence modes. With a typical integration time of 1/15 s the Telecam SL PDD achieves sensitivity better than 0.2 lux. Luminous sensitivity can be increased by one order of magnitude. The endocamera weighs about 160 g and therefore has no need for any holding device. The camera can nicely communicate with the D-Light AF system to synchronize illumination and detection modes. For blue light illumination (both AF and ALA mode) the camera is switched to the blue light mode from the white light mode which is used as for conventional endocameras. The blue light mode allows the extended on-chip-integration as described above and has a specially adapted built-in color balancing function for fluorescence mode to match with the colors that are visually perceived. The complete system is compact and provides flexibility by allowing examination of a fluorescence finding with the white light as often as necessary. Additional image storage devices can be easily configured and added.

Clinical experience is reported in this issue and elsewhere [2,7–9]. The system is compact (Fig. 3), easy to handle, economic and gives brilliant images for all illumination modes. The KARL STORZ AF system is CE-marked and commercially available for the European market and selected countries.

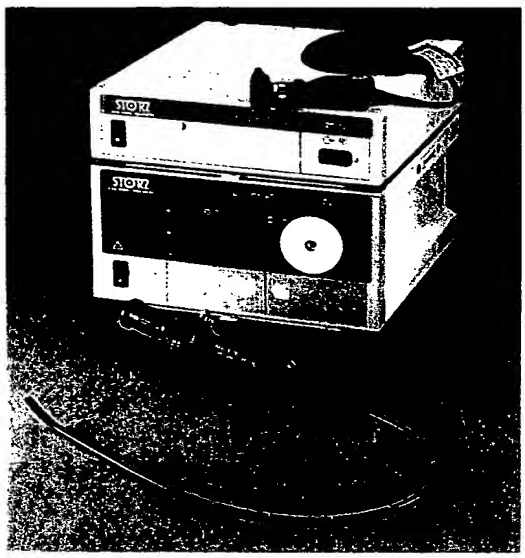

FIGURE 3 Karl Storz fluorescence detection system D-Light AF with fiberbronchoscope 11004 BI and Telecam SL PDD.

Acknowledgements

We kindly thank our clinical and scientific partners Professor Dr. Häußinger, Dr. Stanzel (Fachklinik Munich-Gauting) and PD Dr. Huber (Klinikum Innenstadt, University of Munich) and the University of Munich at Großhadern Laser Research Department with Dr. Baumgartner, Dr. Stepp and Dipl. Biol. Pichler for brilliant work.

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US005891016A

United States Patent [19]

Utsui et al.

[11] Patent Number: 5,891,016

[45] Date of Patent: Apr. 6, 1999

[54] **FLUORESCENCE ENDOSCOPE HAVING AN EXCITING LIGHT FILTER AND A FLUORESCENCE FILTER**

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[73] Assignee: **Asahi Kogaku Kogyo Kabushiki Kaisha,** Tokyo, Japan

[21] Appl. No.: **741,467**

[22] Filed: **Oct. 30, 1996**

[30] **Foreign Application Priority Data**

Nov. 9, 1995 [JP] Japan 7-290854

[51] **Int. Cl.**[6] **A61B 1/06**

[52] **U.S. Cl.** **600/181**; 600/160; 600/476

[58] **Field of Search** 600/109, 181, 600/407, 310, 312, 342, 476, 478

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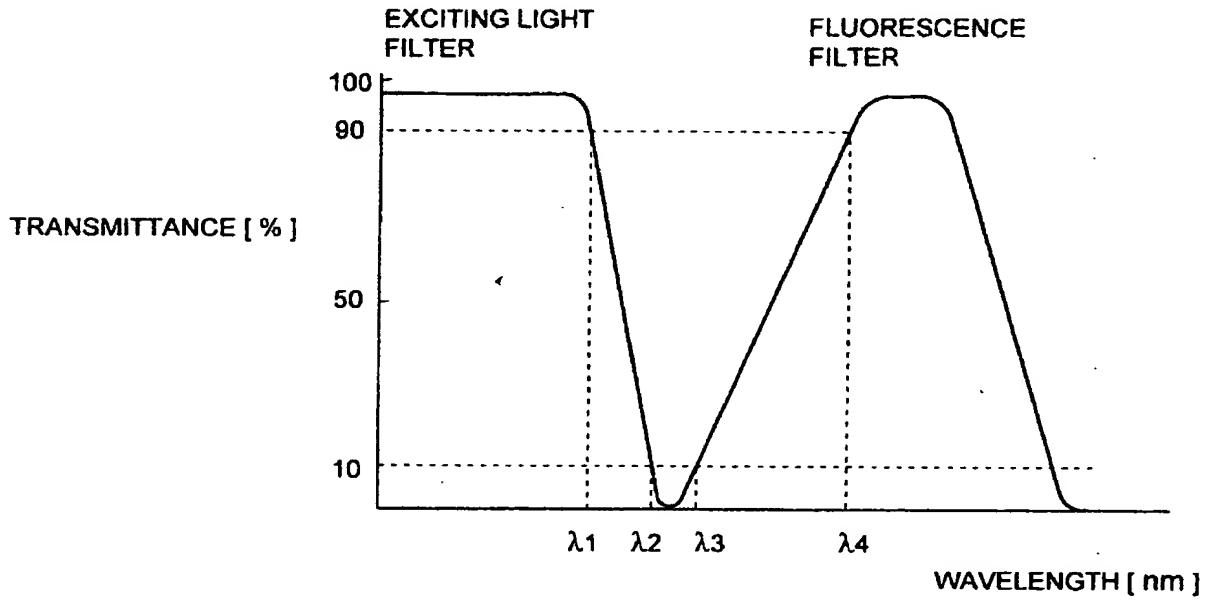
Primary Examiner—John P. Leubecker

Attorney, Agent, or Firm—Kane,Dalsimer,Sullivan, Kurucz, Levy,Eisele and Richard, LLP

[57] **ABSTRACT**

An exciting light filter through which exciting light which is adapted to radiate fluorescence from a living tissue is transmitted, is provided in an illumination light path defined between a light source which emits the illumination light and an object to be observed, in a fluorescence endoscope. A fluorescence filter which permits fluorescence which is radiated from the living tissue due to the illumination by the exciting light and whose wavelength is longer than that of the exciting light to pass therethrough but does not permit the exciting light to pass through the fluorescence filter, is provided in an observation light path defined between the object and a viewing portion in which an image of the object is viewed. There is a difference of 20 nm to 40 nm between the longest wavelength of the wavelength band of which more than 10% are transmitted through the exciting light filter and the shortest wavelength of the wavelength band of which more than 10% are transmitted through the fluorescence filter.

11 Claims, 2 Drawing Sheets



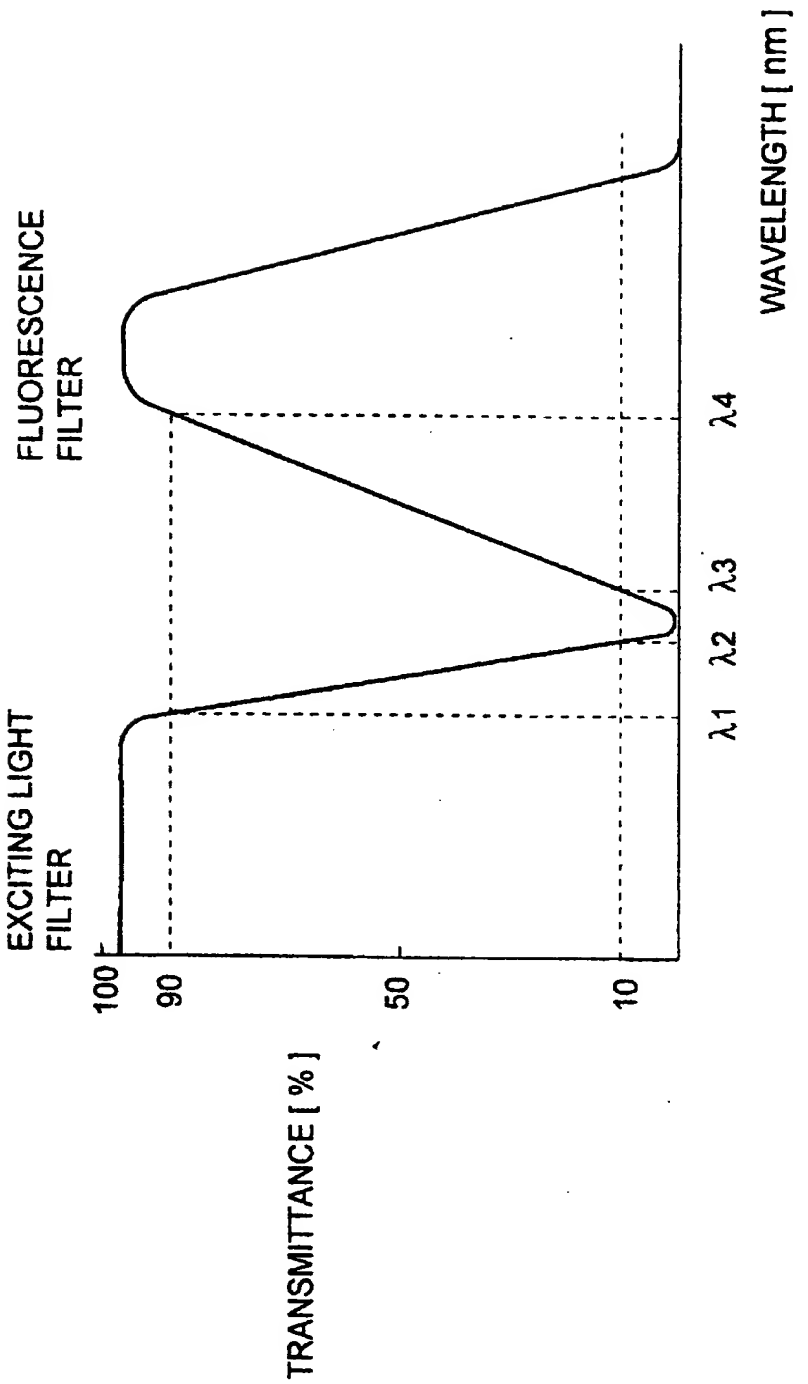
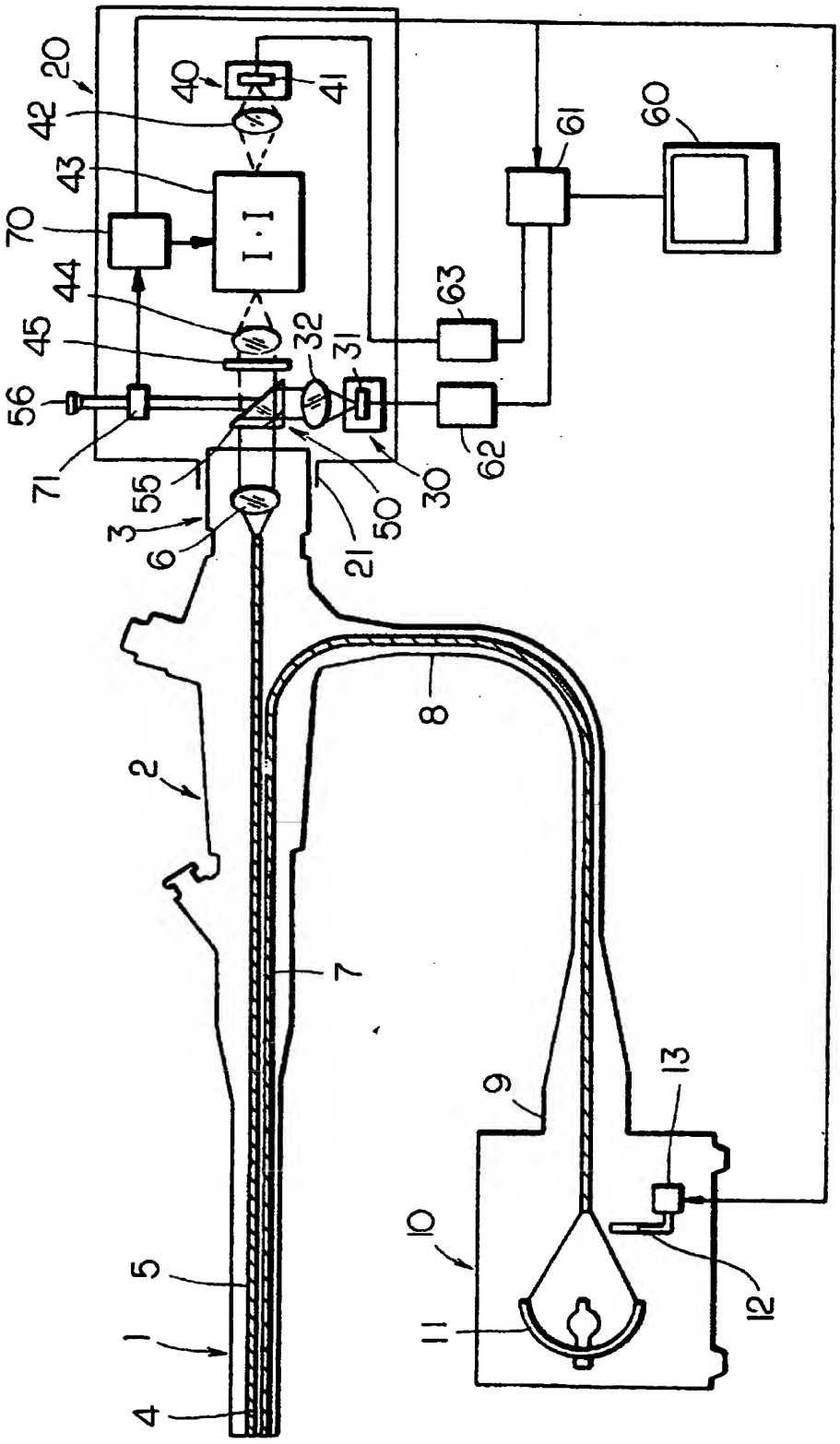

FLUORESCENCE FILTER
EXCITING LIGHT FILTER
WAVELENGTH [nm]
λ1
λ2
λ3
λ4
TRANSMITTANCE [%]
100
90
50
10

FIG. 1


20
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50
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8
2
7
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13
10
5
1
11
12
4

FIG. 2

FLUORESCENCE ENDOSCOPE HAVING AN EXCITING LIGHT FILTER AND A FLUORESCENCE FILTER

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a fluorescence endoscope which is used for directly visually observing a living tissue, using fluorescence which is radiated from the living tissue when visual light is impinged upon the living tissue.

2. Description of the Related Art

In general, in a fluorescence endoscope, an exciting light filter is provided in an illumination light path defined between a light source (lamp) and an object to be examined. Exciting light, adapted to radiate fluorescence from the living tissue, can be transmitted through the endoscope. A fluorescence observing filter (fluorescence filter) is provided in the illumination light path. The fluorescence observing filter permits fluorescence radiated from the living tissue, in response to the exciting light and having a wavelength longer than that of the exciting light, to pass therethrough and does not permit the exciting light to pass through the fluorescence filter.

The wavelength band of the exciting light which excites the living tissue to radiate fluorescence therefrom is approximately 420 nm to 480 nm and the optimal wave length band for the radiation of fluorescence is approximately 450 nm to 475 nm.

On the other hand, the wavelength band of the fluorescence radiated from the living tissue is approximately 480 nm to 600 nm and the peak value of the intensity thereof is obtained at 480 nm to 520 nm, adjacent to the wavelength band of the exciting light.

Since the intensity of the fluorescence produced by the exciting light is extremely small in comparison with the exciting light, even a small amount of exciting light which reaches the observing portion makes it difficult to observe the fluorescence. To this end, the exciting light filter and the fluorescence filter must be produced so that the wavelength band which can pass through the exciting light filter and the wavelength band which can pass through the fluorescence filter do not overlap.

However, since the wavelength bands of the exciting light and the fluorescence are adjacent as mentioned above and they exhibit the peak values of the intensity within limited ranges, if the exciting light transmission range of the exciting light filter is located away from the fluorescence transmission range of the fluorescence filter, the amount of light transmitted through the filter is reduced, so that the intensity of the fluorescence to be observed is weakened, thus resulting in a diagnosis error.

In theory, it is ideal that the exciting light filter and the fluorescence filter are prepared so that the longest wavelength of the light transmitted through the exciting light filter is identical or almost identical to the shortest wavelength of the light transmitted through the fluorescence filter.

The longest wavelength or the shortest wavelength transmitted through the filter refers to the wavelength at which the transmittance of the filter is zero, and hence it is extremely difficult to optically measure the longest wavelength or shortest wavelength. This is the reason that it is very difficult to produce the filter with reference to the longest wavelength that can pass through the exciting light filter or the shortest wavelength that can pass through the fluorescence filter.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a fluorescence endoscope in which, using an exciting light filter and a fluorescence filter, good visual observation using fluorescence can be easily obtained.

To achieve the object of the present invention, there is provided a fluorescence endoscope in which an exciting light filter, through which exciting light which is adapted to radiate fluorescence from a living tissue is transmitted is provided in an illumination light path defined between a light source lamp which emits the illumination light and an object to be observed, and a fluorescence filter which permits fluorescence which is radiated from the living tissue due to the illumination by the exciting light and whose wavelength is longer than that of the exciting light to pass therethrough but does not permit the exciting light to pass through the fluorescence filter is provided in an observation light path defined between the object and a viewing portion in which an image of the object is viewed. There is a difference of 20 nm to 40 nm between the longest wavelength of the wavelength band of which more than 10% are transmitted through the exciting light filter and the shortest wavelength of the wavelength band of which more than 10% are transmitted through the fluorescence filter.

Preferably, the longest wavelength of the wavelength band of which more than 10% pass through the exciting light filter is 455 nm to 460 nm, and the shortest wavelength of the wavelength band of which more than 10% pass through the fluorescence filter is 480 nm to 495 nm.

In the preferred embodiment, in the transmission wavelength band of the exciting light filter on the long wavelength side, the wavelength band in which the transmission changes from 90% to 10% is 12 nm to 20 nm.

Also, in the transmission wavelength band of the fluorescence filter on the short wavelength side, it is preferable that the wavelength band in which the transmission changes from 10% to 90% be 13 nm to 30 nm.

The present disclosure relates to subject matter contained in Japanese Patent Application No. 07-290854 (filed on Nov. 9, 1995) which is expressly incorporated herein by reference in its entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be described below in detail with reference to the accompanying drawings, in which:

FIG. 1 is a graph showing spectral transmission characteristics of a fluorescence endoscope according to the present invention; and,

FIG. 2 is a conceptual view of a fluorescence endoscope according to the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

FIG. 2 shows a whole structure of a fluorescence endoscope in which 1 designates the insertion portion of the endoscope and 2 designates the operation portion connected to the base end of the insertion portion 1. The insertion portion 1 is provided with an (optical) objective system 4 incorporated in the front end thereof to form an image of an object onto the incident end surface of a bundle of image guiding fibers (an image guide fiber bundle) 5. The image guide fiber bundle 5 extends through the operation portion 2 from the insertion portion 1 and reaches an eyepiece portion 3, at the emission end thereof. The eyepiece portion 3 is

provided with an ocular system 6 incorporated therein to provide an enlarged view of the emission end of the image guide fiber bundle 5.

An image of an object located in front of the insertion portion 1 is formed on the incident end surface of the image guide fiber bundle 5 by the objective system 4. The image thus formed is transmitted to the eyepiece portion 3 through the image guide fiber bundle 5. Thus, the object image can be directly and visually observed through the ocular system 6 when the eyepiece portion 3 is not connected to a TV camera unit 20 commonly used for regular light and fluorescence.

A bundle of light guiding fiber (light guide fiber bundle) 7 through which illumination light with which the object is illuminated is transmitted extends in the objective system 4 and the insertion portion 1 and reaches, at the incident end thereof, a detachable connector 9 which is detachably attached to a light source apparatus 10 through a light guide connecting tube 8.

A lamp 11 in the form of a xenon lamp is provided in the light source 10, so that the illumination light emitted from the lamp 11 is converged and made incident upon the light guide fiber bundle 7 at the incident end thereof and is emitted from the emission end of the light guide fiber bundle 7 toward the object to illuminate the same.

An exciting light filter 12 which permits more than 10% of wavelength of for example 380 nm to 460 nm to pass therethrough (i.e., transmittance of more than 10%) is retractably inserted in the illumination light path between the incident end surface of the light guide fiber bundle 7 and the lamp 11 by means of a solenoid 13.

The exciting light filter 12 is retracted from the illumination light path upon normal observation, as shown in FIG. 2 and is inserted in the illumination light path upon an observation using fluorescence (fluorescence observation). The inserted position of the exciting light filter 12 is not shown.

The eyepiece portion 3 can be detachably connected to the regular/fluorescence TV camera unit 20. The latter is provided with a normal image pickup TV camera 30 incorporated therein, which is adapted to pickup the regular (normal) object image transmitted through the ocular system 6 and a fluorescence image pickup TV camera 40 incorporated in the TV camera 20, which is adapted to pickup the fluorescence image transmitted through the ocular system 6. The TV cameras 30 and 40 are integrally formed as a single unit.

The normal image pickup TV camera 30 has a solid-state image pickup device 31 and an imaging lens 32. The fluorescence image pickup TV camera 40 has a solid-state image pickup device 41 and an imaging lens 42. Consequently, when the eyepiece portion 3 of the endoscope is attached to or detached from the regular/fluorescence TV camera unit 20, the endoscope is attached to or detached from the normal image pickup TV camera 30 and the fluorescence image pickup TV camera 40 together.

The fluorescence image pickup TV camera 40 is provided with an image intensifier (I.I.) 43 which remarkably amplifies the intensity of the light transmitted through the ocular system 6. 44 designates the imaging lens to form the object image transmitted through the ocular system 6 onto the incident end surface of the image intensifier 43.

A fluorescence filter 45 which permits more than 10% of wavelength of for example 480 nm to 580 nm to pass therethrough (i.e., transmittance of more than 10%) is provided in front of the imaging lens 44, so that only the light whose wavelength can pass through the fluorescence filter 45 can be made incident upon the image intensifier 43. The light whose wavelength passes through the exciting light filter 12 does not pass through the fluorescence filter 45.

When the light whose wavelength passes through the exciting light filter 12 is emitted toward the living tissue, the normal tissue radiates fluorescence of wavelength which passes through the fluorescence filter 45 but no fluorescence is radiated from, for example, cancer tissue or the like. Consequently, when the exciting light filter 12 is inserted in the illumination light path, the fluorescence radiated from the normal tissue only of the object (living tissue) is made incident upon and intensified by the image intensifier 43.

A light path switching optical system 50 is provided in the front end of the regular/fluorescence TV camera unit 20 to switch the light path of the light transmitted through the ocular system 6 between a first light path connecting with the TV camera 30 and a second light path connecting with the TV camera 40.

The light path switching optical system 50 is comprised of a roof prism 55 which is provided with a reflecting surface inclined at 45° with respect to the optical axis of the ocular system 6 and which is movable in a direction perpendicular to the optical axis of the ocular system 6. The movement of the roof prism 55 is carried out by an operation rod 56.

Consequently, when the roof prism 55 is located on the optical axis of the ocular system 6 as shown in FIG. 2, the image transmitted through the ocular system 6 is reflected laterally by the roof prism 55 and is formed on the solid-state image pickup device 31 of the normal image pickup TV camera 30.

When the roof prism 55 is laterally moved and retracted from the optical axis of the ocular system 6, the image transmitted through the ocular system 6 passes through the fluorescence filter 45 and is formed on the light receiving surface of the image intensifier 43, as indicated by a phantom line in FIG. 2. The light intensity of the image is intensified by the image intensifier 43. Consequently, the image is formed on the solid-state image pickup device 41 of the fluorescence image pickup TV camera 40.

In the illustrated embodiment, there is a single TV monitor 60 to which the image signal output from the regular image pickup TV camera 30 or the fluorescence image pickup TV camera 40 is sent. The selection of the image signals from the TV cameras 30 and 40 is effected by a line selector 61. Numerals 62 and 63 represent controllers for the TV cameras 30 and 40, respectively.

A controller 70 having a micro processor incorporated therein outputs control signals to control the operation of the image intensifier 43, the line selector 61 and the exciting light filter 12, etc., in association with the switching operation of the light path switching optical system 50. A detector 71 detects the switching state of the light path switching optical system 50 and supplies the detection signal to the controller 70.

Consequently, upon normal observation, the exciting light filter 12 is retracted from the illumination light path in the light source apparatus 10, as shown in FIG. 2, so that the object is illuminated by the normal illumination light. Thus, the observed object image is picked-up by the regular image pickup TV camera 30.

On the regular/fluorescence TV camera unit 20 side, the power source of the image intensifier 43 is turned OFF, and the line selector 61 is switched to the normal image pickup TV camera 30. Consequently, the observed normal image formed by the whole wavelength of the visible light is

indicated in the TV monitor **60** in accordance with the image signal output from the solid-state image pickup device **31** of the normal image pickup TV camera **30**.

When the light path switching optical system **50** is switched to move the roof prism **55** in the lateral direction so that the roof prism **55** is retracted from the illumination light path, while keeping the eyepiece portion **3** connected to the regular/fluorescence TV camera **20**, the exciting light filter **12** is inserted in the illumination light path and the power source of the image intensifier **43** is turned ON. Consequently, the line selector **61** is switched to the fluorescence image pickup TV camera **40**.

As a result, the object is illuminated by the illumination light (exciting light) whose wavelength passes through the exciting light filter **12**. The observed image is transmitted through the fluorescence filter **45** and is made incident upon the image intensifier **43**.

Thus, only the light whose wavelength passes through the fluorescence filter **45** is made incident upon the image intensifier **43**. Namely, the fluorescence radiated from the object is only made incident upon the image intensifier **43**, so that the intensified fluorescence image is picked-up by the solid-state image pickup device **41** of the fluorescence image pickup TV camera **40** and is indicated in the TV monitor **60**.

<EXAMPLE 1>

FIG. **1** shows spectral transmission characteristics of the exciting light filter **12** and the fluorescence filter **45**. In the downward curved portion of the transmission wavelength band of the exciting light filter **12** on the long wavelength side in FIG. **1**, the wavelength at which the transmission is 90% is λ1 and the wavelength at which the transmission is 10% is λ2, respectively. Also, in the raised portion of the transmission wavelength band of the fluorescence light filter **45** on the short wavelength side in FIG. **1**, the wavelength at which the transmission is 10% is λ3 and the wavelength at which the transmission is 90% is λ4, respectively.

In general, for a filter, it is practically extremely difficult to measure the wavelength at which the transmission is 0% or 100%, but the wavelength at which the transmission is 10% or 90% can be easily optically measured.

In Example 1 in which the exciting light filter **12** and the fluorescence filter **45** having the following optical characteristics were used, a light and clear fluorescence observation could be carried out.

λ1: 435 nm

λ2: 455 nm

λ3: 495 nm

λ4: 525 nm

<EXAMPLE 2>

In Example 2, the exciting light filter **12** and the fluorescence filter **45** having the following optical characteristics were used. Similarly to Example 1, a light and clear fluorescence observation could be carried out.

λ1: 440 nm

λ2: 458 nm

λ3: 488 nm

λ4: 513 nm

<EXAMPLE 3>

In Example 3, the exciting light filter **12** and the fluorescence filter **45** having the following optical characteristics were used. Similarly to Example 1, a light and clear fluorescence observation could be carried out.

λ1: 448 nm

λ2: 460 nm

λ3: 480 nm

λ4: 493 nm

As can be understood from the above discussion, according to the present invention, since the exciting light filter and the fluorescence filter are formed such that there is a predetermined difference between the longest wavelength of the wavelength band of which more than 10% pass through the exciting light filter and the shortest wavelength of the wavelength band of which more than 10% pass through the fluorescence filter, the transmission wavelength bands do not overlap. Moreover, according to the present invention, the exciting light filter and the fluorescence filter whose transmission wavelength band is located adjacent or very closely to the transmission wavelength band of the exciting light filter can be easily prepared, based on the precise measurement of the wavelength.

What is claimed is:

1. A fluorescence endoscope in which living tissue is illuminated by exciting light and radiated fluorescence light from said living tissue is received and said radiated fluorescence wavelength is longer than that of said exciting light, said fluorescence endoscope comprising:

a light source lamp, which emits illumination light;

an illumination light path defined between said light source lamp and an object to be observed;

a viewing portion in which an image of said object is viewed;

an observation light path defined between said object and said viewing portion;

an exciting light filter through which exciting light which is adapted to radiate fluorescence from a living tissue is transmitted, said exciting light filter being provided in said illumination light path; and

a fluorescence filter, which permits fluorescence which is radiated from said living tissue due to illumination by said exciting light to pass therethrough but does not permit said exciting light to pass therethrough, said fluorescence filter being provided in said observation light path;

wherein there is a difference of 20 nm to 40 nm between said longest wavelength of said wavelength band of which more than 10% are transmitted through said exciting light filter and said shortest wavelength of said wavelength band of which more than 10% are transmitted through said fluorescence filter.

2. The fluorescence endoscope according to claim **1**, wherein said longest wavelength of said wavelength band more than 10% of which pass through said exciting light filter is 455 nm to 460 nm.

3. The fluorescence endoscope according to claim **2**, wherein said shortest wavelength of said wavelength band of which more than 10% pass through said fluorescence filter is 480 nm to 495 nm.

4. The fluorescence endoscope according to claim **1**, wherein said transmission wavelength band of said exciting light filter on said long wavelength side, in which said transmission changes from 90% to 10% is 12 nm to 20 nm.

5. The fluorescence endoscope according to claim **4**, wherein in said transmission wavelength band of said fluorescence filter on said short wavelength side, in which said transmission changes from 10% to 90% is 13 nm to 30 nm.

6. The fluorescence endoscope according to claim 1, wherein on said long wavelength side of said exciting light filter, at which said transmission is 90% said transmission wavelength λ1 is 435 nm, and at which said transmission is 10% said transmission wavelength λ2 is 455 nm.

7. The fluorescence endoscope according to claim 6, wherein on said short wavelength side of said fluorescence filter, at which said transmission is 10% said transmission wavelength λ3 is 495 nm, and at which said transmission is 90% said transmission wavelength λ4 is 525 nm.

8. The fluorescence endoscope according to claim 1, wherein on said long wavelength side of said exciting light filter, at which said transmission is 90% said transmission wavelength λ1 is 440 nm, and at which said transmission is 10% said transmission wavelength λ2 is 458 nm.

9. The fluorescence endoscope according to claim 8, wherein on said short wavelength side of said fluorescence filter, at which said transmission is 10% said transmission wavelength λ3 is 488 nm, and at which said transmission is 90% said transmission wavelength λ4 is 513 nm.

10. The fluorescence endoscope according to claim 1, wherein on said long wavelength side of said exciting light filter, at which said transmission is 90% said transmission wavelength λ1 is 448 nm, and at which said transmission is 10% said transmission wavelength λ2 is 460 nm.

11. The fluorescence endoscope according to claim 10, wherein on said short wavelength side of said fluorescence filter, at which said transmission is 10% said transmission wavelength λ3 is 480 nm, and at which said transmission is 90% said transmission wavelength λ4 is 493 nm.

* * * * *

REFERENCE 7

Diagnostic and Therapeutic Endoscopy, Vol. 5, pp. 65–70
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Development of the Autofluorescence Endoscope Imaging System

RENSUKE ADACHI[a,*], TETSUYA UTSUI[a] and KOICHI FURUSAWA[b]

[a] *Research and Designing Department, Medical Instrument Division;* [b] *Research and Development Division, Asahi Optical Co., Ltd. 2-36-9 Maenocho Itabashi-ku, Tokyo 174, Japan*

It is a well known fact that light emitted at a specific wavelength induces fluorescence in the human body. This kind of fluorescence is called autofluorescence. The application of autofluorescence diagnosis, on the other hand, is a more complicated system designed to detect faint autofluorescence inherent in tissues/cells. We have adopted this autofluorescence diagnosis method and developed a new autofluorescence endoscope imaging system called the SAFE-1000. Normal mucosa emitting autofluorescence appears green on the monitor, while abnormal mucosa shows a dark image caused by the lack of autofluorescence.

Keywords: Autofluorescence, Bronchoscope, Carcinoma *in situ*, Image intensifier

1. INTRODUCTION

Endoscopic application of fluorescence for medical detection of carcinoma has recently attracted considerable attention. These spectroscopic techniques can be classified into two basic types. One method is called photodynamic diagnosis (PDD) [1,2,3] using chemicals called photosensitizers that react to various wavelengths of light. The other method is autofluorescence diagnosis, hereafter referred to as AFD, employing inherent tissue/mucosal autofluorescence without the use of additional photosensitizers.

PDD using tumor-affinitive photosensitizers is the easier of the two techniques to apply. However, after intravenous administration of these chemical photosensitizers, a patient must remain in a darkened environment until these light sensitive chemicals have metabolized to a safe level. Actual diagnosis via this method is somewhat more difficult due to the fact that fluorescence from the photosensitizers interferes with the autofluorescence from normal mucosa.

The application of AFD, on the other hand, is a more complicated system designed to detect faint autofluorescence inherent in tissues/cells. Since it does not require the use of any photosensitizers, it produces fewer side effects. Furthermore, it can be performed in conjunction with conventional endoscopy. With safety concerns being the primary

* Corresponding author.

factor, Asahi Optical Co., Ltd. has adopted this AFD method and developed a new autofluorescence endoscope imaging system called the SAFE-1000.

It is a well known fact that light emitted at a specific wavelength induces fluorescence in the human body [3–9]. This kind of fluorescence is called autofluorescence. Typically, a greater amount of autofluorescene occurs with normal mucosa and little fluorescene is exhibited by abnormal tissue. Applying this same principle, Pentax has developed a new autofluorescence endoscope imaging system (Pentax SAFE-1000) without the application of any photosensitizing agents or use of any laser.

By utilizing the same white light used in conventional endoscopy through a pass filter that allows passage of only specific wavelengths, the SAFE-1000 collects blue light and delivers it endoscopically to mucosa to excite tissue autofluorescence. By detecting and amplifying the faint autofluorescence at a green wavelength from normal mucosa, a highly sensitive video camera incorporated into the SAFE-1000 can show the intensified green autofluorescent image on a color monitor. However, for abnormal tissue, the autofluorescence at the green wavelength is significantly diminished, making it appear dark on the video monitor. It can then be easily distinguished from the more intense autofluorescence obtained from normal mucosa [2].

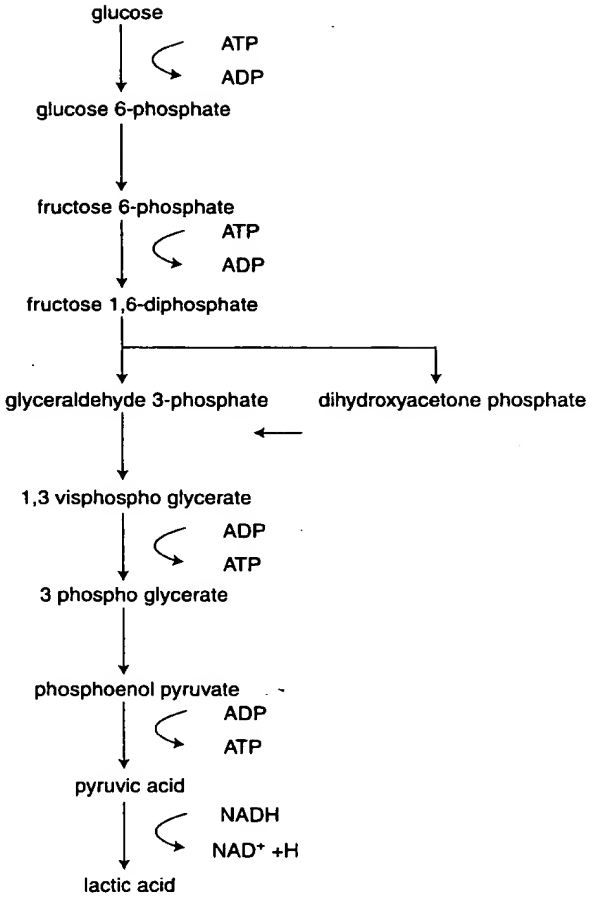

FIGURE 1 The overproduction of lactic acid through glycolysis.

2. EXPERIMENTAL MODEL

Otto Warburg was a German biochemist who discovered that there are considerable biochemical differences between normal cells and cancer cells [10]. For instance, he found that cancer cells produce more lactic acid than normal cells. This phenomenon was confirmed both *in vivo* and *in vitro* studies. Several investigations have been performed on the overproduction by cancerous cell of lactic acid, called the Warburg effect. Though the entire process has not been completely defined, it has been confirmed that this overproduction (of lactic acid) is generated by glucose through a glycolysis pathway (Fig. 1).

This increase in the overproduction of lactic acid through glycolysis is due not so much to decreased enzymatic action but to an increase in fermenting activity. The fact that tumors consume more glucose than is usually required for normal growth indicates that there is some malfunction in the mechanism at which the cells control the speed of absorption of glucose. It has actually been confirmed that the cellular absorption speed of glucose is increased in abnormal cells.

The following common substances found in the human body are known to exhibit tissue autofluorescence (Table I).

TABLE I Fluorescent substances in the human body

Fluorescent substance	Exitation light wavelength (nm)	Fluorescent wavelength (nm)
Tryptophan	280	340
Collagen	325	380
Elastin	410	440
NADH	365	470
Flavin	440	520
Porphyrin	400	630, 690

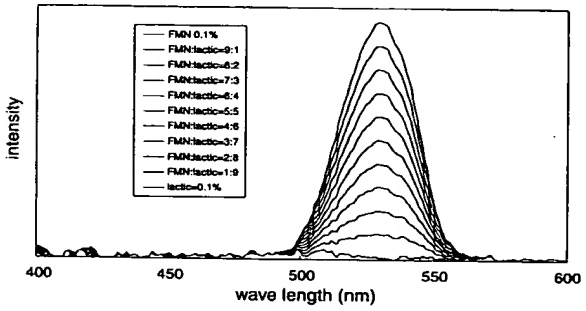

FIGURE 2 Fluorescence spectrum by changing the ratios of FMN and lactic acid.

The substances that participate in the AFD are not specified. In our study, we noted that when the fluorescent substance is mixed with other substances, the peak wavelength of the fluorescence is not changed and is merely attenuated.

In this study, flavin and its derivative, flavin mono nucleotide (FMN), have been selected because flavin is associated with cellular/biochemical metabolism and fluoresces in the green wavelength. Based upon the Warburg effect, it is believed that the different metabolic processes between normal mucosa and tumors might have some effects on the spectral emission of their autofluorescene. We have therefore developed the following experimental model (Fig. 2).

Figure 2 shows the fluorescence intensity exhibited by several mixtures of FMN and lactic acid due simply to changing their proportions. At a molecular level, FMN emits autofluorescence while lactic acid does not. Depending upon the ratios in each mixture, the fluorescence intensity of each mixture varies. Flavin and its derivatives such as FMN or FAD are enzymes or coenzymes that are widely distributed throughout the human body and are strongly associated with oxidation–reduction reactions. Consequently, it is the molecular make-up and their different metabolic processes which produce the different relative densities of autofluorescent and non-autofluorescent substances. This seems to be one of the factors that would then contribute to the different intensities in autofluorescence inherent in various tissues. Moreover, it is a well-known fact that blood flow increases in tumors and cancerous tissues. Blood can be considered as a non-autofluorescent substance in this case, because it does not emit any autofluorescence at the observed wavelength. Therefore, the increased blood flow in tumors seems to be one of the primary factors that could cause different intensities of autofluorescence in various types of tissues. As a result, it would appear that the SAFE-1000 could aid in the detection of tumors by identifying metabolic differences, due to changes in cellular abnormalities, via autofluorescence.

3. PENTAX SAFE 1000 (A NEW AUTOFLUORESCENCE ENDOSCOPE IMAGING SYSTEM)

3.1. System Configuration

(1) Target Area: bronchial tubes.
(2) Equipment (Fig. 3):
  (1) A bronchofiberscope (Pentax FB-18RX).
  (2) An excitation light source (Pentax LX-750AF) incorporating a 75 W xenon lamp and an excitation filter (EX filter). The EX filter is automatically inserted to or removed from the light path with the changeover switch on the camera box. Through the EX filter, the light source delivers excitation blue light for antofluorescence endoscopy. Without the filter, the light source delivers white light for conventional endoscopy.

(3) A video camera incorporating
- an endoscopic TV camera with a 410000-pixel 1/2″ CCD;
- a fluorescence TV camera;
- light path changing optics (prism);
- a fluorescence filter (FL filter);
- an image intensifier which amplifies faint fluorescence signal thousands of times;
- a changeover witch on the TV camera box. The switch changes the camera for endoscopic or fluorescence use and simultaneously inserts or removes the EX filter to or from the light path;
- an image intensifier control switch (AUTO FL CONTROL) on the TV camera box. The switch can adjust the brightness of fluorescence images.

(4) An image intensifier controller (Pentax SAFE-1000c) which
- processes fluorescence images (frame average addition of two-frame, four-frame, eight-frame or live image with the recursive filter);
- changes the monochrome fluorescence signal to the G signal;
- adjusts the brightness and contrast of the fluorescence images.

(5) Peripherals
- a monitor;
- a video cassette recorder;
- a video printer.

3.2. Operation

(1) Conventional Endoscopy

By setting the changeover switch on the camera box to "NORMAL", conventional endoscopic procedure can be performed. The 75 W xenon lamp in the

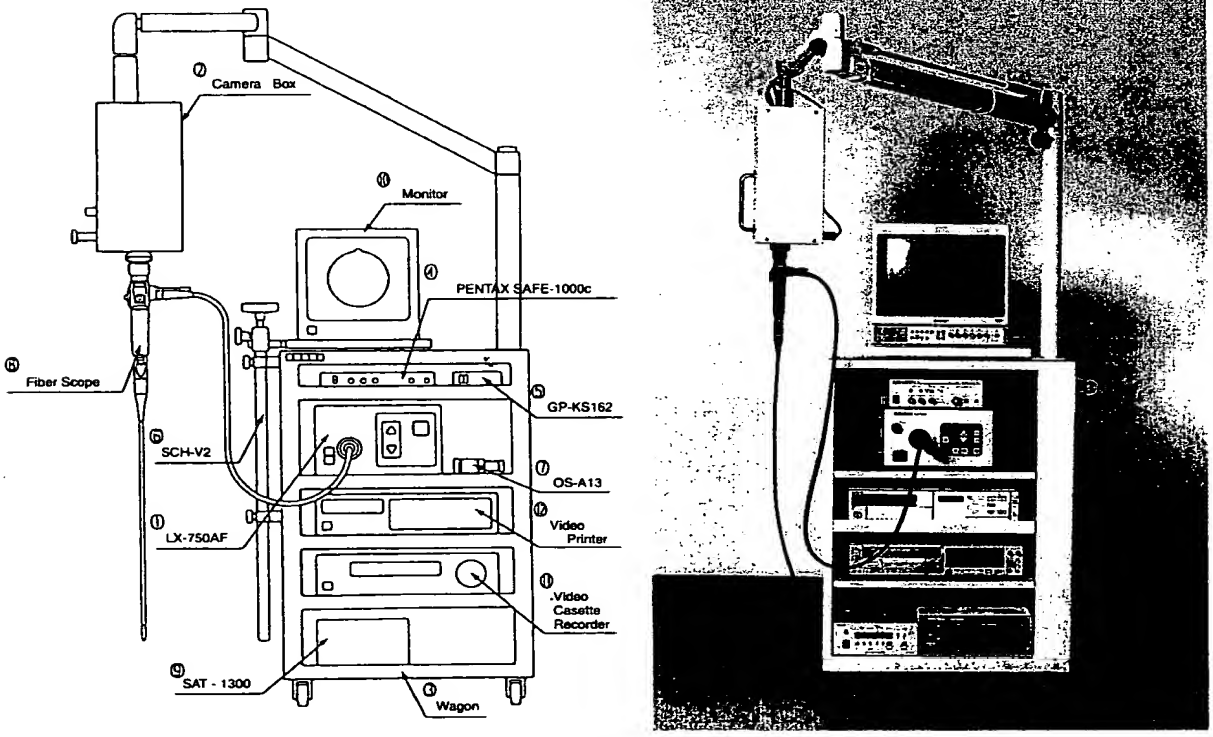

FIGURE 3 Pentax SAFE-1000.

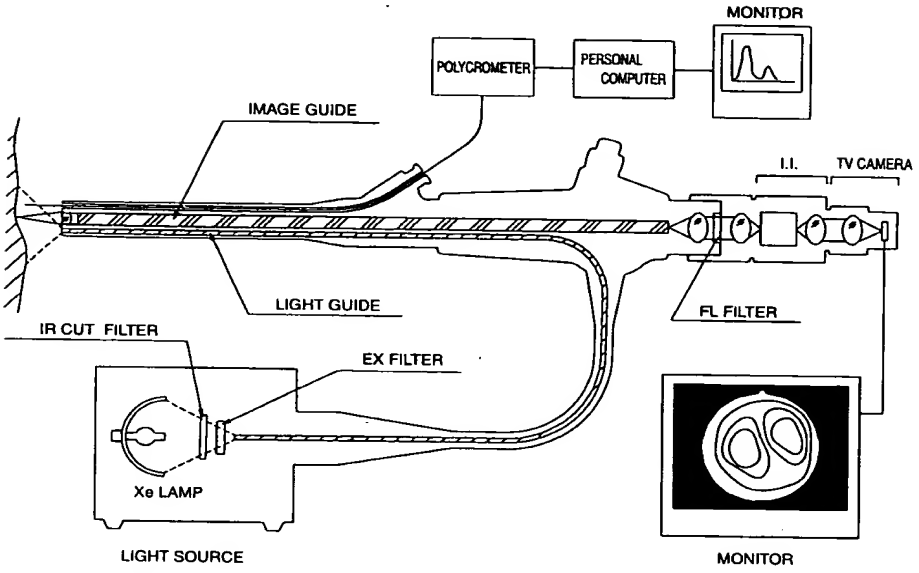

FIGURE 4 The optical system of endoscopic autofluorescence imaging.

light source emits white light. Infrared light is eliminated by the infrared (IR) cut filter. Bypassing the EX filter, white light is transmitted via a light guide to the target area. Images obtained with an objective lens are transmitted via a fiberoptic image guide back to the eyepiece of the endoscope and guided through the prism to endoscopic TV camera.

(2) Autofluorescence Endoscopy

By setting the changeover switch on the camera box to "AUTO FL", an autofluorescence endoscopic procedure can be performed (Fig. 4). The white light from the 75 W xenon lamp is passed by the IR cut filter and then by the EX filter which specifically passes 420–450 nm excitation light. The excitation light is then transmitted via a light guide to the target area.

Images obtained with an objective lens are transmitted via a fiberoptic image guide back to the eyepiece of the endoscope and guided through the prism to the FL filter which specifically passes 490–590 nm fluorescence signal. The selected signal is then amplified by the image intensifier thousands of times, and by means of a video camera, appears as fluorescent images on the monitor. Normal mucosa emitting autofluorescence appears green on the monitor, while abnormal mucosa shows a dark image caused by the lack of autofluorescence, which allows for easier recognition of carcinoma in situ [11].

(3) Features

- Fluorescence observation without using any photosensitizers;
- Detection of early cancers not superficially visible;
- Safer and less expensive without using lasers;
- Possible to use conventional endoscopes;
- A compact and space-saving system;
- Easy to changeover between conventional and fluorescent images, which enables moreaccurate diagnosis.

4. CONCLUSION

The autofluorescence endoscope imaging system, Pentax SAFE-1000, should aid physicians in the ability to recognize areas of early cancer and

squamous metaplasia and in the detection of precancerous lesions which cannot be identified by conventional endoscopy. Compared to other fluorescence techniques requiring photosensitizers, this method is more appealing to and less traumatic for patients.

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Review article

Fluorescence bronchoscopy for early detection of lung cancer
A clinical perspective

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Abstract

The conventional method of bronchoscopy has only a 30% sensitivity to detect early stage cancer in the central airways. For patients with positive sputum cytology who clearly harbor early cancers, repeat and lengthy sessions of bronchoscopies are required for accurate localization of these lesions. This leads to a significant delay in obtaining the diagnosis, postponing an appropriate treatment and reduces the chance for cure. There are valid reasons for improving the detection rate of early stage lung cancers. The number of individuals at risk forms a large population, the outcome of patients treated with early stage cancer has been shown to be better and bronchoscopic treatments, e.g. photodynamic therapy and electrocautery, are currently alternatives for surgical resection. Finding more early stage cancers by screening the population at risk and accurate staging to enable treatment at the earliest stage feasible, may improve the dismal prognosis of many patients. This article deals with the clinical background and current problems in early detection of lung cancer and discusses our expectations regarding new developments in bronchoscopy for early detection, accurate staging and treatment of lung cancer.

Keywords: Bronchoscopy; Cancer; Lesions

1. Introduction

Lung cancer is a global epidemic and number one killer among all cancers. Morbidity is high and cure rate is only 13%. Many lung cancer patients are diagnosed in a relatively advanced stage, precluding radical resection [1]. Surgery provides the best chance for cure if the tumor can be radically resected and when no lymph node or distant metastases is present. In only 25% of the population can the tumor be resected and half are ultimately cured. Even after curative treatment of their primaries, patients remain at risk for local

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recurrence, distant metastasis and subsequent primaries [2]. Treatment modalities, such as cisplatin-based chemotherapy and radiotherapy for unresectable lung cancer, are becoming part of the treatment strategy, achieving significant improvement of quality of life, longer median survival time and higher survival rate, if compared to palliative care alone [3,4]. Gene susceptibility for both smoking and developing lung cancer make the management of the target population quite complex [5,6]. Many lung cancer patients also suffer from smoking related diseases, such as COPD and their cardiovascular status is often poor. Reduced physical fitness hamper our abilities to apply the best treatment strategies because of treatment related morbidity. Smoking and cancer susceptibility of the individuals at risk also explains why, after previous curative treatment of cancer primaries in the aero-digestive tract, many remain at risk of developing subsequent primaries [7]. The rate of second primary cancer after a non-small cell lung cancer is in the order of 1–4%. Earlier study has shown a cumulative incidence for second primary lung cancer of 4% per year [8].

Invasive squamous cell cancer in the central airways gradually develops from normal mucosa and may be multifocal, the so-called field cancerization [9]. Research to reverse gradual progression of normal tissue towards cancer, e.g. chemoprevention studies and gene therapy, is currently one of the major activities in medical oncology [10,11]. Inhaled carcinogens may cause cumulative genetic damage to the entire mucosa of smokers and ex-smokers. Genetic abnormalities gradually develop until morphological changes, such as dysplasia and carcinoma in situ, become morphologically apparent [12–14]. Patches containing 200–400 cell clones have recently been found to harbor early molecular changes similar to those seen in tumor cells [15]. Various molecular damage may indicate heterogeneity in the pathways of development towards cancer of multiple cells' clones in the bronchial mucosa. The finding that these clonal patches may only contain 90,000 cells makes it quite obvious that conventional diagnostic methods, such as chest X-rays, CT scan and bronchoscopy, cannot be regarded as promising tools and are therefore inappropriate for early detection. These clones are far below the detection threshold of conventional diagnostic tests. It is therefore important to develop more sensitive and accurate diagnostic methods that can be applied in the target group for the detection of early stage lesions.

Surgical resection is proven to provide the best chance for cure. However, it is not always feasible because of the relative wasteful method of resection of healthy lung parenchyma. Many patients have limited pulmonary function suffering from COPD [6]. As parenchyma sparing techniques, such as surgical bronchoplasty and video-assisted thoracoscopic surgery, require clinical expertise, there will always be a need for less morbid alternatives for the standard surgical approach [16]. In early stage cancer of the central airways, fluorescence system has been shown to increase the yield of finding pre-invasive lesions [17,18]. Central and proximal airway branches can be inspected with the fiberoptic bronchoscope during the conventional white light bronchoscopy and the additional use of the fluorescence imaging equipment has been shown to be feasible in clinical practice. Tumor margins are clearly visible and taking biopsy specimens becomes more precise. Techniques, such as thermal lasers, high frequency electrocautery, photodynamic therapy, cryotherapy and brachytherapy, can then be applied in a more accurate manner [16,19]. Further study is needed to look for the most optimal method of fluorescence detection. The development of various imaging systems for optical biopsy may help us to understand carcinogenesis without the necessity to biopsy and thus, affect the natural history of disease [20].

Early diagnosis is aimed to find cancer at the earliest stage before patients become symptomatic. Hence, treatment of patients with occult cancer should not cause too much morbidity deteriorating quality of life. Many patients with early stage cancer can greatly benefit from less invasive and morbid treatment approaches, which offer a better quality of life. Less invasive diagnostic and therapeutic methods are indispensable elements for success in a lung cancer' screening program and increase the value of early detection.

2. The need for fluorescence bronchoscopy

Woolner et al. showed that the finding rate of carcinoma in situ in patients with positive sputum cytology is only 29% [8]. Two-thirds of these 'radiographically occult' cancers are indeed only a few millimeters thick. The conventional method of white light bronchoscopy relies on the visual judgement of the bronchoscopist [21]. Expertise is important for the correct judgement of these minute lesions. Mucosal thickening, swelling, granularity, nodules and polyps are quite obvious. However, redness, paleness, lack of luster, vascular engorgement, disruption of mucosal folds, loss of clarity, edematous change, small vesicles, tiny necrotic material and the fact that the suspicious area easily bleeds are sometimes too subtle for easy recognition. The bronchoscope should be maneuvered elegantly without bruising the bronchial mucosa. The accessibility of the entire bronchial tree is also limited by the size of the bronchoscope [22]. Regions distal to the segmental bronchi are more difficult to examine. It is understandable that minute lesions that are smaller than the size of the flexible biopsy forceps are easily missed. The individuals at risk may also suffer from bronchitis, whose bronchial mucosa is often inflamed, swollen and easily bruised during bronchoscopy. To differentiate normal from suspicious lesion may be quite difficult. Tumor may also infiltrate beneath the mucosa and abnormal tissue may be present as skipping lesions. Sampling error in taking biopsy for histology examination can thus lead to a false negative result, requiring repeat and lengthy examinations for accurate staging.

Sato et al. have shown the difficulty in detecting and localizing carcinoma in situ with the conventional white light bronchoscopy [23]. In 180 patients with 200 occult cancers, 527 bronchoscopic sessions were needed to confirm the diagnosis. An average of three lengthy sessions per patient up to 45 min was required. Brushing all segments to collect representative samples was performed, while 175 lesions were located proximal to the sub-segmental bronchi within the visible range of the bronchoscope. 'False negative' findings led to an average delay of 29.2 months until the definite diagnosis could be made and the exact location of early stage cancer was pinpointed. Another study showed that in 61% of the cases, the first attempt of white light bronchoscopy was successful [24]. Fortunately, 44 out of the 51 (88%) lung cancers were still found to be early stage. It is obvious that despite some expertise, tedious examinations are generally required to ultimately locate all pre-neoplastic lesions in the individuals at risk. A screening study as part of the daily clinical routine in a large population, based on these considerations will not be feasible.

It is also generally accepted that microscopic examination by the pathologists using the WHO-criteria is the gold standard [25]. However, significant disagreement between pathologists does exist. Even among experienced pathologists, full agreement of classifying malignant bronchial biopsy specimens is lacking. Sub-classification of non-small cell carcinoma using the standard WHO-classification showed a κ of 0.25. Intra-individual and inter-individual variability in classifying pre-neoplastic lesions does exist [26]. Recent data showed that specimens that were histologically classified as normal, do contain early molecular changes [12–15]. Quantitative image cytometry and molecular biological studies have revealed changes that are beyond the detection threshold of the pathologists [27–29].

In 1924, Policard reported that sarcoma fluoresces red when illuminated with Wood's light [30]. Ever since, more selective tumor diagnostic methods have been developed. Photodynamic therapy has emerged as a 'selective' tumor therapy [31]. Fluorescence imaging technique for the detection of early stage lung cancer is based on the physics of photon tissue interaction [32]. The optical characteristics of tissue, such as absorption coefficient, scattering, anisotropic factor, the presence of fluorophores and the kinetics of photosensitizers, have been extensively investigated to develop 'tumor selective' imaging methods [33]. Spectral imaging techniques exploited the different emission spectra of tumor tissue compared to normal tissue. The presence of endogenous fluorophores in tissue is the basis of autofluorescence imaging [17,18,32]. Drug-induced fluorescence detection, by adding exogenous

photosensitizers to the neoplastic tissue, is aimed at enhancing contrast [34]. The lack of drug selectivity, complex pharmacokinetics and the fact that some sensitizers may cause skin photosensitivity, make the clinical application of drug induced fluorescence more complex. Spectroscopic images obtain the entire spectral images of the target tissue within a wavelength of interest. Optical coherence tomography recording two-dimensional images of the target tissue at one specific wavelength is currently under full investigation, as well as micro-confocal optical scanning microscopy to study carcinogenesis at the cellular level [35,36]

The autofluorescence technique for the lung, the LIFE® system (Xillix®, Vancouver, BC, Canada), uses a Helium-Cadmium laser for excitation at $\lambda = 442$ nm [17,32]. The emission spectrum is captured by two CCD cameras and processed through a fluorescence collection sensor and an optical multi-channel analyzer. Real time digitized images are acquired by the ratios of red to green ($\lambda = 630$ to 520 nm) fluorescence emissions, also for correction of the distance during bronchoscopic examinations to compensate movements due to breathing and cough. Special algorithms are based on data of in vivo and in vitro analysis and Monte-Carlo simulation modeling [32]. Digitized images reflect the in vivo fluorescence signals of the bronchial mucosa, exploiting differences in autofluorescence spectra between the suspicious area (moderate dysplasia, severe dysplasia, carcinoma in situ, early stage cancer) and normal tissue. During LIFE examination, the suspicious area appears red-brownish, while normal mucosa appears as green. The D-light system (Storz®, Tüttlingen-Germany), uses non-coherent ultraviolet to blue ($\lambda = 380–460$ nm) light to excite a broad emission spectra of the different chromophores in tissue [34]. A 300-W Xenon filtered lamp is used. At the tip of the bronchoscope, ± 80 mW is emitted (versus 10–20 mW monochromatic light $\lambda = 442$ nm of the LIFE system). In combination with some reflectance of blue light from the tissue, the D-light produces bluish images for normal areas and darkened images in case of malignancies.

Recently, the SAFE® 1000 autofluorescence system (Pentax®, Asahi Optical Tokyo, Japan) has also become commercially available [37]. Excitation uses a Xenon-lamp ($\lambda = 420–480$ nm) and the camera contains a fluorescence filter as well as an image intensifier.

It is also possible to add photosensitizers, such as porphyrin derivatives, to enhance image contrast of the target tissue [34]. Based on the relative selectivity of the photosensitizing agents for abnormal tissue, high power photons' excitation and higher photon yield due to greater emission may abolish the need for expensive laser light excitation and the use of CCD cameras.

Fluorescence imaging method has been shown in two large multicenter trials, in which large numbers of individuals have been studied with review of histology specimens, to be more sensitive than conventional bronchoscopy alone to detect pre-neoplastic lesions [38,39]. Recent data indicate that the increased sensitivity of using the fluorescence technique is not biased by the sequence of examinations, either using the conventional or the fluorescence equipment first [40]. Fluorescence bronchoscopy therefore provides us with an important tool to assist the bronchoscopist, in addition to the conventional method for imaging early stage cancers. Biopsy can be performed with more precision reducing errors in sampling representative specimens.

3. Which system do we need to improve the detection rate of early lung cancers?

An imaging system should be practical for use on a daily basis for the purpose of screening, either alone or in combination with routine diagnostic procedures, such as white light bronchoscopy. Especially for a lung cancer screening program, due to the large number of individuals, a versatile, non-expensive and accurate diagnostic tool is absolutely necessary. Experiences in using the LIFE system showed that the additional procedure extended the routine white light examination by 15 min [41]. It can be performed on an outpatient basis under local anesthesia. However, diagnostic bronchoscopy in itself is not a screening tool. Bronchoscopy is performed as a diagnostic procedure and as part of the tumor staging

process. Therefore, fluorescence bronchoscopy is currently used as an addition to improve staging of intraluminal tumor [42]. It remains to be seen whether its ability to detect 'invisible' early stage cancers can justify a more liberal approach for screening at large.

Diagnostic accuracy, however, should not rely on the high sensitivity rate alone. Especially in the lung cancer population, 90% of the individuals at risk will not develop cancer and 96% of those after previous treatment of a cancer primary will not develop subsequent tumors. Initial screening should first exclude those who are not at risk. Hence, the current interest in early biological markers, serum markers and sputum cytology screening [12–15,33]. By excluding the majority of individuals who are not at risk at the first pass, additional and follow-up investigations can then be concerted on the cohort of patients who are really at greatest risk.

Moreover, sensitivity of a technique is a relative issue. True positive findings can only be related to the total positive lesions that were sampled, as it is impossible to excise the entire tracheobronchial mucosa to find all pre-neoplastic lesions, except in the case of a post-mortem study [43,44].

The classical study performed by Auerbach in 1961 was aimed to investigate the relation of smoking to changes in bronchial epithelium. Step sections of the entire tracheobronchial tree of 402 male patients had been carried out, up to 208 sections per individual. The true presence of especially carcinoma in situ had been established and was strongly related to the smoking behavior and related to the presence of lung cancer. A meticulous analysis was performed on the average of 46.4 sections' materials per lung cancer patient and 52.3 sections per subject without lung cancer. The magnitude of this study can never be compared with an auto-fluorescence bronchoscopy study. Early primary invasive carcinomas were found in 23 of the 956 sections showing atypical cells in the entire specimen, compatible with carcinoma in situ (CIS). These CIS lesions had an average depth of about five cells (4–38 cells). In early primary invasive carcinoma, 61% of the lesions had depth abnormalities of greater than or equal to six cells' layer. So, these lesions are indeed only a few cell layers thick. These findings are in accordance with other studies, [8,9,43–48], indicating the importance of detecting micro-invasive cancer at the earliest stage possible, thereby improving the chance for cure if treated. Non-smokers did not harbor any carcinoma in situ [43,44]. The incidence of carcinoma in situ is correlated to the number of cigarette packs smoked. CIS was found in 75% (27/36) of the subjects who smoked greater than or equal to two packs per day and 83% (52/63) in lung cancer subjects. Percentage of sections found with CIS was proportionally related to the smoking habit and strongly supports field cancerization. Subjects also had increased number of sections with CIS depending on their smoking habit, 11.4% in those who smoked greater than or equal to two packs per day and 15% in the cohort of patients who died of lung cancer. In a later study, Auerbach reported a significant decrease of the percentage of abnormalities found, showing a much lower rate of abnormalities [44]. In heavy smokers who died in the period 1970–1977, the rate was found to drop from 22.5 to 2.2%.

Interestingly, the risk of developing second primary has been recently reported to be 1–4% after previous non-small cell cancer primaries [7,8]. The natural history of CIS and its rate of progression to micro-invasive carcinoma have recently been reported [48,49]. We performed a follow-up study in patients with CIS to study the natural history of disease [48]. A panel of pathologists has confirmed the presence of CIS in the initial biopsy specimens. A wait and see policy has been adopted and repeated bronchoscopy every 3–4 months was performed. Patients were not treated until progression to invasive carcinoma was documented, or worsening of the macroscopic appearance during white light and autofluorescence bronchoscopy was documented. Current data indicate that all but one lesion progressed to microinvasive cancers and the last patient was also treated because of the progression of the bronchoscopic appearance. The rate of progression to invasive cancer is higher than the 23% progression rate previously reported at 3 months follow-up by another group [49]. In our study, mechanical biopsy was unlikely to cause ' spontaneous regres-

sion', as the pathologists confirmed that CIS was seen up to the margin of the biopsy specimens. The high rate of CIS in the study of Auerbach and the high rate of progression in our study are intriguing numbers and seem not to reflect the epidemiological data. One may then expect a much higher rate of primaries ($\approx$15% of heavy smokers) and second primaries (1–4%) in the target population, if CIS is a one way process towards invasive cancer. It is possible that many CIS reported before, in the absence of any meticulous review by a panel of expert pathologists, are not 'true CIS' because of the lack of a gold standard [26]. Therefore, 'misclassification' of pre-neoplastic lesions as CIS may explain the discrepancies of the rate of progression and 'spontaneous regression' in many previously published studies. Only additional longitudinal studies together with molecular biology markers may provide the definite answer of how the natural history of disease really is. It is also possible that the quite advanced age of the individuals at risk, who are found with CIS, may not allow us to study the rate of progression because many of them will 'prematurely' die because of non-cancer related diseases, preventing invasive cancers to be clinically significant.

Inclusion criteria of the population studied, e.g. male–female ratio, smoking status, the numbers of biopsies taken, sampling errors and the lack a gold standard for histology, are important factors to be taken into account in analyzing and comparing sensitivity, specificity and accuracy of several fluorescence detection and screening programs [50].

So far, the LIFE-programs have included the largest number of individuals and lesions investigated [38–40]. Based on the two large multicenter trials, with mandatory review of the histology specimens by expert pathologists, the use of the LIFE systems was shown to significantly increase the sensitivity of detecting neoplastic lesions, e.g. moderate dysplasia, severe dysplasia and CIS [38,39].

Data from our own institution have been analyzed in more details [41]. A learning period in using the LIFE® system is shown in Table 1. Inconsistent histology classifications of the specimens were striking (Table 2). There was a κ of 0.548 between the panel of two expert pathologists involved in the early detection program and the staff pathologist [26]. The agreement regarding moderate dysplasia was much worse, with a κ of 0.02.

Table 1
The learning curve in Academic Hospital Vrije Universiteit of autofluorescence bronchoscopy with the LIFE system for early detection of pre-neoplastic lesions

	Patients no. 1–48	Patients no. 49–95
Sensitivity (%)	67 (8/12)	86 (24/28)
Specificity (%)	67 (82/123)	71 (112/158)
Positive predictive value (%)	16 (8/49)	34 (24/70)
Negative predictive value (%)	95 (82/86)	97 (112/116)

Specimens were reviewed for histology classifications of moderate, severe dysplasia and CIS by a panel of two expert pathologists.

Our follow-up study also indicates that the LIFE® system may detect lesions that ultimately progress, morphologically, questioning the value of the initial definition 'false positives' [48]. In the past, computer image cytometry analysis and immunostaining with hnRNP have also been shown to provide a much better positive predictive value than conventional cytology [27–29]. Our longitudinal study underscores the need for molecular

Table 2
Comparison of the initial histology classification of carcinoma in situ (CIS) lesions by a staff pathologist versus the definite classification by a panel of two expert pathologists involved in the multicenter trial

	Panel positive	Panel normal	Total
Staff pathologist positive	20	25	45
Staff pathologist normal	3	295	298
Total	23	320	343

A κ of 0.548 for CIS was found. (the κ for moderate dysplasia was 0.02!).

Table 3
Examples of longitudinal follow-up biopsies of two locations after the initial 'false positive' fluorescence

Months	6	7	10	16	20
WLB	N	N	S	N	N
AB	N	S	N	S	S
Histology	Normal	Normal	Severe dysplasia	Normal	Carcinoma
Months	14	18	21	24	31
WLB	N	N	N	S	N
AB	S	S	S	N	S
Histology	Inflammation	Hyperplasia	Normal	Not sufficient	Severe dysplasia/CIS

Lesions ultimately progress to a more invasive stage and conventional histology seemed to 'lag' 13 and 16 months, respectively. WLB, white light bronchoscopy; AB, autofluorescence bronchoscopy; N, normal; S, suspicious for pre-malignancy.

markers in studying carcinogenesis. The specimens initially classified as normal ('false positive' fluorescence) became malignant after 13 and 17 months follow-up (see Table 3). Recent data by Park et al. showed molecular abnormalities to be quite extensive and multifocal in histologically normal bronchial specimens [15]. In 68% (13/19) of the patients, the specimens showed at least one abnormal focus among the seven chromosomal regions analyzed. Heterogeneity was seen in the changes of the molecular pattern and these were not necessarily identical with those found in tumor clones. A more homogeneous allele specific loss was found in materials sampled with the LIFE® system in their previous study. This may suggest that abnormal fluorescence characteristics may represent a more malignant cohort of lesions, than in samples collected by random biopsy alone. Unfortunately, clonal patches removed by bronchoscopic biopsy were quite small, < 1.5 mm in diameter [45]. Bronchoscopic biopsy may result in complete removal of the lesions. About 50% of these patches were found to be smaller than the size of the flexible biopsy forceps. Twenty-seven of the 69 paired biopsies obtained at 6-months intervals showed one or more molecular changes in the initial biopsies. However, 86% had no abnormality after re-biopsy and 24% lacked the initial changes found after re-biopsy. Sequential biopsies may therefore radically resect the majority of these cell clones, a bystander curative effect. So, the natural history of these lesions cannot be properly studied because of the 'curative diagnostic' biopsies.

The lack of understanding in carcinogenesis and the lack of gold standard for histology classification of pre-neoplastic lesions are important drawbacks. We do not know whether 'dysplastic' lesions and early molecular changes are expressions of an irreversible development towards cancer [51]. We still do not have full understanding of the correlation between fluorescence characteristics, morphology and molecular changes. False negative fluorescence rate is found to be in the order of 15–20%, again based on current histology classification being the gold standard. Current data cannot tell how clinically important 'positive fluorescence' findings are. Do they represent non-specific changes, e.g. inflammation? What is the clinical impact of squamous metaplasia? Does fluorescence imaging miss a proportion of pre-neoplasia that may become malignant? Or are these lesions representing non-specific 'non-malignant' clones? What is the dynamism of bronchial mucosa when continuously exposed to irritants during life? How different are these changes biologically from carcinogenesis? The 50% rate of complete removal by biopsy may hamper any longitudinal study on carcinogenesis. Optical coherence tomography and endoscopic microconfocal scanning microscopy may allow us to study the natural history without the necessity for taking biopsy [46,50]. However, before the value of 'optical biopsy' can be defined, long-term clinical and molecular studies should be conducted and longitudinal tissue sampling remains necessary.

We have calculated the cost of LIFE-bronchoscopy in finding pre-neoplastic lesions. In the first period of our prevalence study, 113 patients at risk were recruited. The tariff of white light bronchoscopy in the Netherlands is Euro 81,-. We have performed 209 sessions to detect 101 moderate, severe dysplasias and one CIS. The LIFE-system found 21 additional lesions at the cost of Euro 814,- per lesion. The importance of these lesions in the ultimate management of the patients with early stage cancers can only be answered after longer follow-up.

Despite one negative study [52], a significant increase in sensitivities of fluorescence technique for detecting pre-neoplastic lesions have been reported [38,39]. Possible biases due to the sequence of bronchoscopic examinations and the expertise of the individual bronchoscopist, have recently been ruled out [40].

Tables 4 and 5 compiled some data regarding studies with large number of individuals studied and comparison of available data using several fluorescence imaging systems. The different sensitivity rates of the white light bronchoscopy from different centers may also indicate differences in the populations studied and differences in bronchoscopists' 'visual threshold' [50,53]. Thousands of biopsies are needed, as only a limited percentage of these lesions are pre-neoplastic. Only large numbers of biopsy can provide us with the 'true denominator' to calculate absolute sensitivity, specificity and accuracy. The lengthy process of carcinogenesis also requires long follow-up and not prevalence studies.

4. What can be expected from current data about the future of fluorescence bronchoscopy in a lung cancer screening study?

Bronchoscopists clearly need assistance to accurately localize minute pre-invasive lesions [21,23]. Finding CIS which progress to microinvasive carcinoma can timely allow the bronchoscopist to cure patients by applying effective local treatment, such as electrocautery or photodynamic therapy [54,55]. Early stage squamous cell cancer ≤ 1 cm in diameter and ≤ 3 mm deep is N0 cancer and can be cured with bronchoscopic treatment as an alternative for surgical resection [16,46,47,62]. This is justified, as the size of 'early stage' cancer is the most important determinant of 'cure' using any intraluminal bronchoscopic technique. Caution remains necessary because tumor staging by bronchoscopy is relatively inaccurate, if compared to surgical exploration and resection. The use of high resolution CT, fluorescence bronchoscopy and endobronchial ultrasound have been reported to improve staging's accuracy [42,56,57].

There is a resurgence of interest in lung cancer screening by sputum cytology, chest X-rays or low dose spiral CT [58–60]. Stage migration may result in a higher cure rate because more patients will be saved by surgical resection, video-assisted thoracoscopic surgery and bronchoscopic treatments. The ultimate yield of screening, however, will strongly depend on the population studied. The cohort of patients at risk of developing centrally located squamous cell cancer and peripheral adenocarcinoma may differ from one country to

Table 4
Compiled data for the sensitivity rate of previous studies using autofluorescence LIFE system for early detection

	Routine bronchoscopy	LIFE bronchoscopy	Routine and LIFE
Lam et al. 1993 [18]	48	72	
Lam et al. 1994	39	79	84
Ikeda et al. 1997	36	96	100
Nakhosteen et al. 1997	41	94	
Lam et al. 1998 [38]	9		56
Venmans et al. 1998	59	80	85

Studies with more than 100 high risk patients were included. Full references upon request.

Table 5
Sensitivity rates and positive findings for moderate, severe dysplasia and carcinoma in situ of the different autofluorescence systems: LIFE, D-light and SAFE

System (*n* biopsies)	Routine white light	Autofluorescence system	% Positive
LIFE Xillix (4173)	40	86	15.2
D-light Storz (771)	25	83	3.2
Safe Pentax (157)	21	79	3.2

Differences may reflect differences in: the populations studied, e.g. male:female ratios, smoking index, i.e. definition of 'at risk', 'visual threshold' of the bronchoscopists, numbers of biopsies taken, sampling biopsy errors, inconsistencies of histology classifications. Special references with regard to this table, except the last two references:

- Lam et al., [18,38];
- Thiberille et al., [39];
- Khanavkar et al. Basic principles of LIFE autofluorescence bronchoscopy. Results of 194 examinations in comparison with standard procedures for early detection of bronchial carcinoma – Overview [in German] Pneumologie 1998;52:71-76;
- Vermijlen et al. Detection of preneoplastic lesions with fluorescence bronchoscopy (FB) ERS Berlin meeting 1997: Suppl. Abstract no. 2741 pp 425S;
- Venmans et al. ref. no. 57 and Early detection of preinvasive lesions in high risk patients. A comparison of conventional flexible and fluorescence bronchoscopy J Bronchology 1998;5:280-283;
- Yokomise et al. Clinical experience with lung imaging fluorescence endoscope(LIFE) in patients with lung cancer. J Bronchology 1997;4:205-208;
- Ikeda et al. Early localization of bronchogenic cancerous / precancerous lesions with lung imaging fluorescence endoscope. Diagn Ther Endosc 1997;3:197-201;
- Haussinger et al. Autofluorescence detection of bronchial tumors with the D-Light/AF. Diagn Ther Endosc 1999;5:105-112 [D-Light Storz® system];
- Kakihana M et al. Early detection of bronchial lesions using system of autofluorescnece endoscopy (SAFE) 1000. Diagn Ther Endosc 1999;5:99-104 [SAFE 1000 Pentax® System].

another. The issue of quality of life has to be considered in the survivors. There is a significant shift in lung cancer incidence towards adenocarcinoma in many countries, justifying the use of low dose spiral CT. Kaneko et al. showed that 14/15 adenocarcinoma found were Stage I [59]. However, the detection rate for peripheral lung cancer by low-dose spiral CT was 0.43%, as 3457 examinations had been performed in the population at risk. One has to take into account the social, economical and psychological impact of false positives and also the ultimate effect of 'overdiagnosis' bias [61]. This may end in treating cancer that is not going to be clinically important nor causing any morbidity to the individual at risk.

Fluorescence bronchoscopy can play a significant role if squamous cell cancer is still an important cohort in a lung cancer population. This may translate in finding more central early stage lesions that are amenable for bronchoscopic treatment as an alternative for surgery.

Recently, the impact of screening has been reported by Koike et al. [53]. Stage migration was achieved for Stage I cases from 55.1 to 59.6%. There is a marked increase in finding of X-ray negative hilar 'occult' cancers, from 0 to 40 cases. Thirty-three patients were cured (82.5% cure rate). Fluorescence bronchoscopy can be adopted to find these lesions at the earliest stage possible, allowing patients to be treated with curative intent, e.g. with electrocautery or photodynamic therapy.

Therefore, if a larger cohort of squamous cell cancer exists, more benefit can be expected from sputum cytology screening and autofluorescence bronchoscopy. In the Netherlands for example, squamous cell type comprises $\pm 35\%$ of all lung cancer. If all early stage squamous cancers can be detected and treated on time, a cure rate of 82.5% may improve total cure rate from $< 13\%$ to $\pm$ 29%. However, a compliance rate of 75% was recently reported, which may reduce the efficacy of screening to a cure rate of $\leq 21.6\%$ [61]. So,

there is not much room for complacency in screening the population at risk. All available means and methods should be diligently and efficiently applied to reduce lung cancer mortality and to provide survivors with good quality of life. One may expect that the autofluorescence technique together with other techniques can improve treatment efficacy as well in using photodynamic therapy, lasers or electrocautery [42,56]. Whether drug induced fluorescence can improve the ± 85% relative sensitivity rate significantly, remains to be seen. One has to consider the practical aspects of increased costs and time in a large population study, the burden of skin photosensitivity and the issue of poor selectivity of photosensitizer molecules. Efforts should focus on changing the mortal attitude towards smoking and to counter the fatalistic attitude of physicians and patients about early detection and treatment of lung cancer [6].

5. Summary

Fluorescence bronchoscopy significantly increases the finding rate of pre-neoplastic lesions and has helped us to better understand carcinogenesis. The exact benefit for patient management is not yet clear because carcinogenesis is a complex phenomenon. Current criteria of histology classifications cannot be reliably used for staging early stage lesions. Therefore, we cannot ascertain the clinical impact of early detection with fluorescence bronchoscopy today. Dr Slaughter wrote in 1953: 'detailed knowledge of the natural history of this disease is of some importance'. At the brink of the new millenium, we still do not understand why only some individuals develop lung cancer and who is really at risk among the so many smokers and ex-smokers. Longitudinal study of the individuals at risk and knowledge about the dynamic changes of the bronchial mucosa are necessary before any definite conclusion can be drawn. Suffice to say that fluorescence bronchoscopy has helped us to detect what was previously 'invisible'. Having less ability before to look for early stage lesions systemically, we currently do not know how to interpret this paradox of finding dysplastic lesions. Due to the lack of knowledge about carcinogenesis and the lack of gold standard in histology classifications, a cautious approach regarding early intervention for patients with pre-neoplastic lesions is necessary. Sputum cytology screening with molecular biology analysis and other avenues should be explored towards developing less invasive procedures for screening the population at risk. In combination with less morbid treatment approaches, intervention at the earliest stage possible may guarantee better quality of life and improve the impact of lung cancer screening.

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[Lung autofluorescence. Preliminary study of two systems without laser illumination or photosensitization]

[Article in French]

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When illuminated with a certain wavelength, lung tissue emits a weak fluorescence. The fluorescence from normal tissue is different from that from diseased tissue. This technique can thus be used for early diagnosis of precancerous lesions. Technical manipulations are required to amplify the fluorescence signal. Numerous studies have used the LIFE (Lung Imaging Fluorescent Endoscope) to study lung diseases. This system of detection uses a laser helium-cadmium excitation source; the principal photosensitizing agents are hematoporphyrin derivatives. The cost, bulkiness and secondary effects have limited diffusion of this type of photodynamic diagnostic system. We have used 2 systems (SAFE 1000 Pentax and D-LIGHT Storz) successively in a prospective study to analyze autofluorescence of lung tissue without laser illumination or photosensitization. Fifty-four patients were selected for their risk factors. We were able to detect one case of severe dysplasia but did not identify any case of carcinoma in situ. Among 18 cases, 16 were evident with white light. Two were detected with autofluorescence used to orient biopsies while white light had only revealed a common inflammatory aspect. Thirty-nine low-grade lesions (metaplasia, dysplasia) were found. The systems are easy to use. Lower cost would allow more widespread use of these systems currently reserved for risk populations. These techniques broaden the scope of interventional endoscopy.

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Seeing the Invisible

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The dismal cure rate of lung cancer patients and the stage shift hypothesis, i.e. finding and treating early cancer at the N0 stage, have propelled the interest for early intervention in the individuals at risk. First of all, one has to define the population at risk to prevent the obligation for screening all heavy smokers, as only about 10% of them will develop lung cancer [1]. The next steps are to have accurate diagnostic tests to identify individuals harboring early cancer lesions and apply a cost-effective curative treatment method that preserves quality of life. Only then, one may succeed to counterbalance the many potential burdens associated with lung cancer screening.

For squamous cell cancer, early localization of these minute lesions by conventional white-light bronchoscopy alone may lead to significant delays, counterproductive to stage shift [2]. Early squamous cell cancer develops gradually in a stepwise fashion from normal bronchial epithelium, through the different grades of pre-neoplasia towards cancer [3]. Auerbach et al. [3] reported carcinoma in situ to be only 5–6 cell layers thick, thus several millimeters in size, easily missed using white-light bronchoscopy alone. Lam et al. [4] were the first to show the improved sensitivity of the autofluorescence bronchoscopy (AFB) imaging system for the detection of pre-neoplastic lesions (LIFE®, Xillix, Richmond, Canada) due to the reduced green fluorescence of 'abnormal' tissue. Debates about the value of AFB have been fuelled by several factors: e.g. risk estimation and number of the individuals studied, expertise of the bronchoscopist, consistency of pathologist's reports or the number of biopsies taken [5, 6]. A recent study by Hirsch et al. [7], in which the sequences of the bronchoscopists and the examinations (AFB first or white-light bronchoscopy first) have been randomized for ruling out expertise and memory biases, respectively, definitely confirmed the superior sensitivity of AFB. It is important to realize that only relative sensitivity has always been reported, as not all neoplastic lesions in the entire bronchial mucosa can be sampled bronchoscopically compared to Auerbach's postmortem study [3, 5].

In this issue of *Respiration*, Herth et al. [8] compared two fluorescence-imaging systems, LIFE versus the D-light® (Storz, Tuttlingen, Germany; actually a fluorescence-reflectance system [5]) in 332 patients at risk. They ultimately found 62 meta- or dysplasias, 12 severe dysplasias and 11 carcinomas in situ in 127 specimens (67% relative sensitivity). In only 5 cases was there a difference in image classification between the two imaging systems. Both systems yielded comparable results, i.e. no significant differences in finding or missing (pre-)neoplastic lesions, and the D-light is easier to handle, leading to a shorter average examination time of 8 versus 12 min for LIFE.

The size of a current fiberoptic bronchoscope, however, does not allow inspection beyond the 5th–6th order of the bronchial tree. So, AFB can only be applied for the inspection of the mucosa of the central airways mainly for studying squamous cell carcinogenesis [1, 7, 9, 10].

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AFB allows us to detect previously invisible, early and minute mucosal changes to study the natural history of cancer lesions. High-resolution, accurate and cost-effective imaging systems that exploit the use of minimally invasive procedures are needed as many individuals at risk already suffer from chronic obstructive pulmonary disease and from a poor cardiovascular status due to their smoking habit. They cannot tolerate long-duration and burdensome invasive interventions. Every link in the total strategy of early intervention, including the use of bronchoscopic techniques for early detection, localization and treatment, should be seen as an effort to apply the most cost-effective minimally invasive approach to further reduce screening-related morbidity and mortality. Still, we need more studies to prove that the stage shift hypothesis will indeed lead to a significant reduction in lung cancer mortality, due to potential biases of overdiagnosis and overtreatment in a lung cancer screening setting [11].

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United States Patent [19]

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[45] Date of Patent: *Aug. 8, 2000

[54] **FLUORESCENCE DIAGNOSIS ENDOSCOPE SYSTEM**

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[*] Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

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[63] Continuation of application No. 08/531,921, Sep. 21, 1995, abandoned.

[30] **Foreign Application Priority Data**

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Sep. 21, 1994	[JP]	Japan	6-226522
Sep. 21, 1994	[JP]	Japan	6-226523
Sep. 21, 1994	[JP]	Japan	6-226524

[51] **Int. Cl.**[7] **A61B 1/06**

[52] **U.S. Cl.** **600/160**; 600/178; 600/109

[58] **Field of Search** 600/109, 118, 600/160, 178, 181, 407, 476, 478

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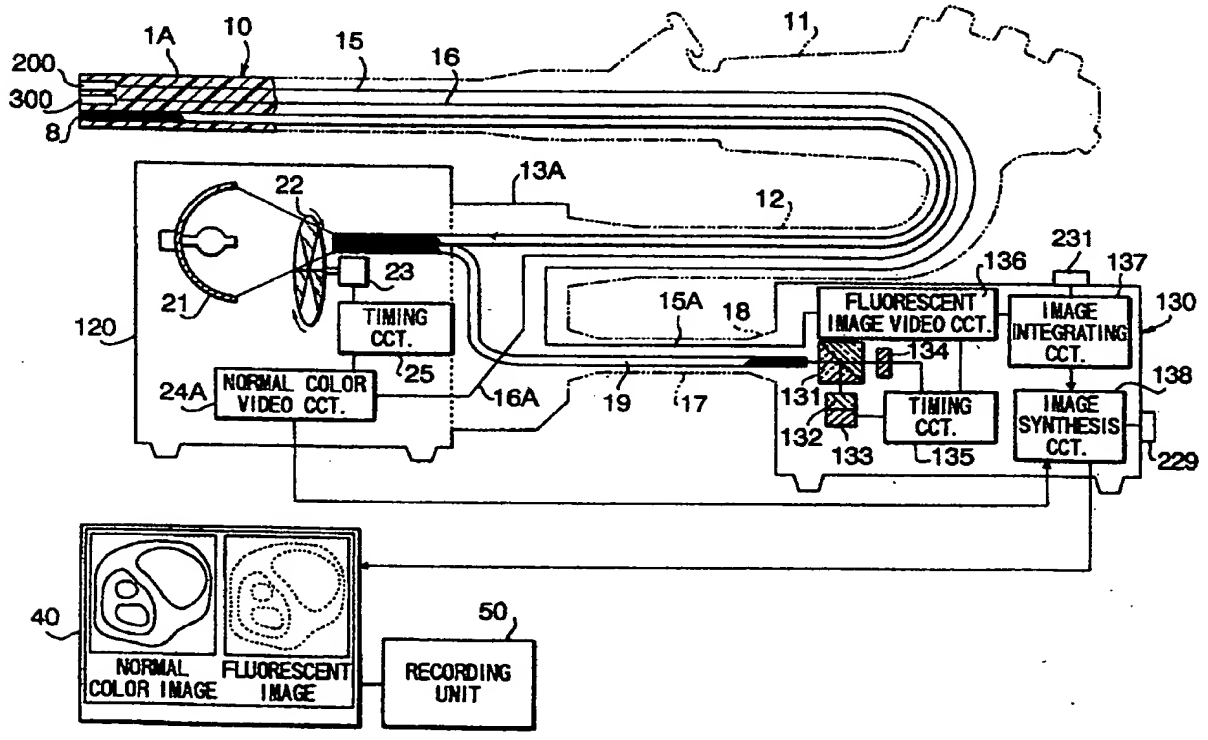
Primary Examiner—John P. Leubecker

Attorney, Agent, or Firm—Greenblum & Bernstein, P.L.C.

[57] **ABSTRACT**

A fluorescence diagnosis endoscope system used to observe human tissue is provided. The system has a light source for illuminating the human tissue with normal light and excitation light having a certain wavelength range. The human tissue fluoresces in response to illumination with the excitation light. The system further has a pair of image receiving elements for outputting image signals corresponding to received optical images. A filter which transmits only light produced by the fluoresced human tissue is provided in front of one image receiving element. The image signals output by the image receiving elements are selectively processed and transmitted to a display.

14 Claims, 13 Drawing Sheets



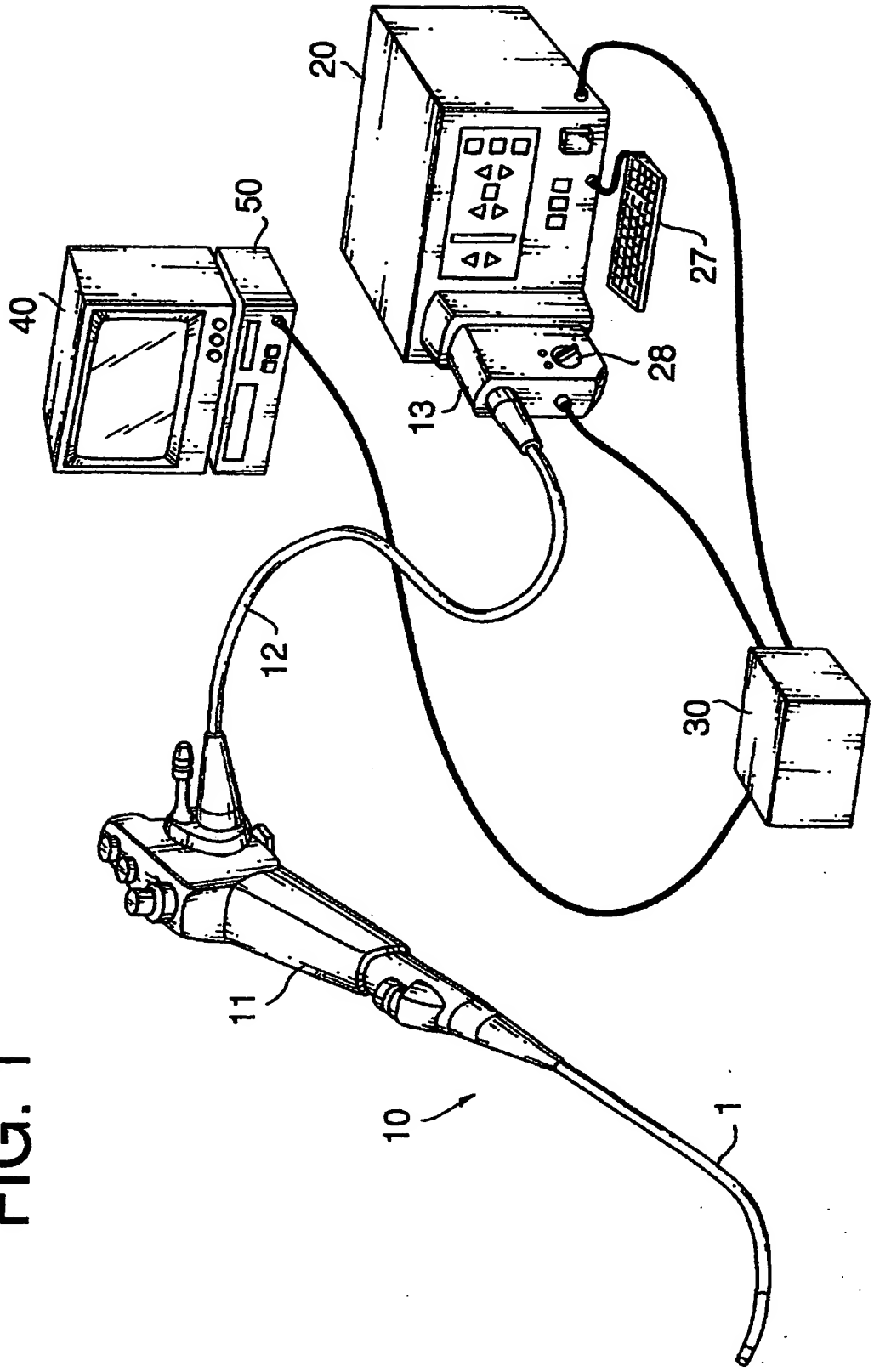
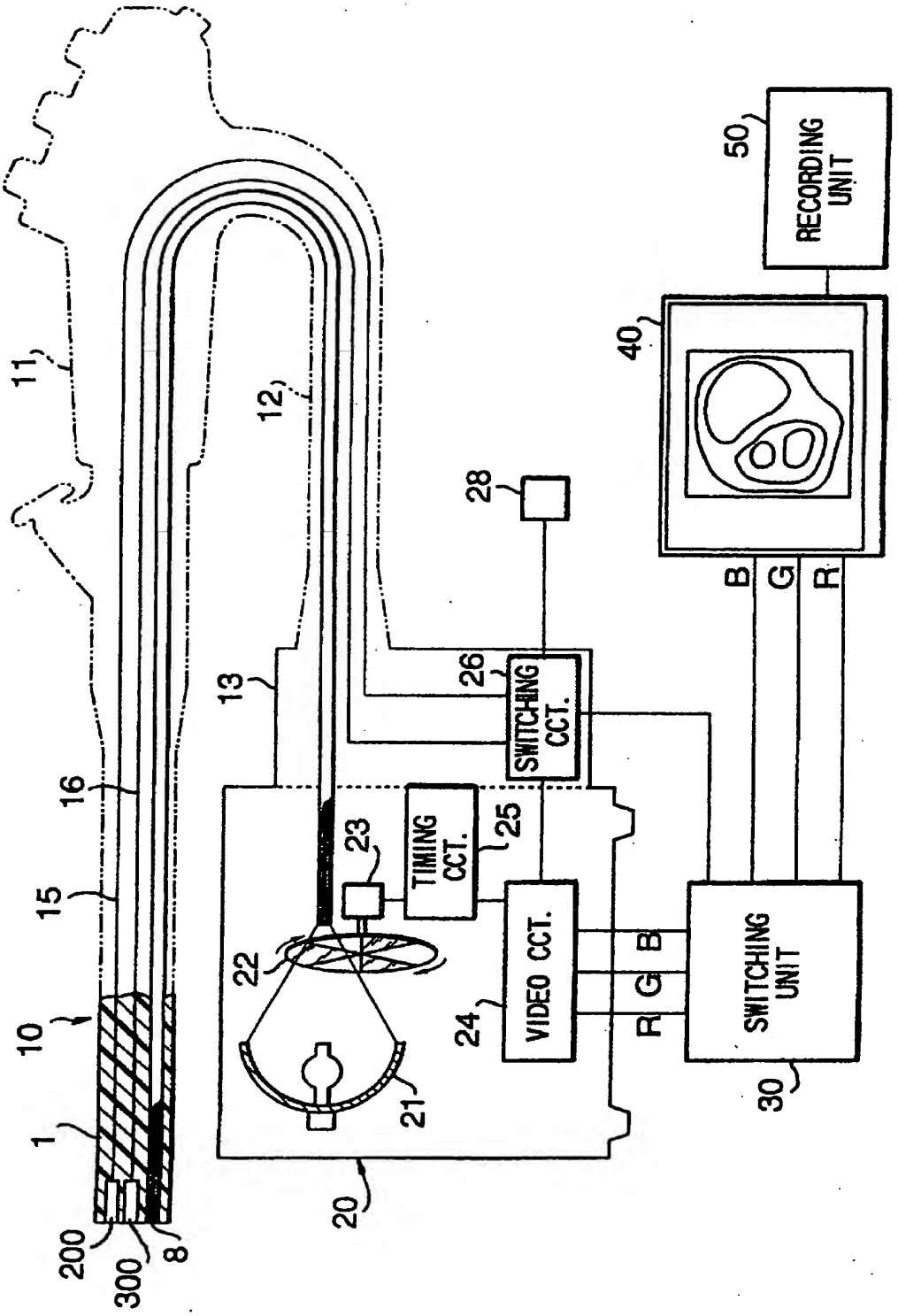


FIG. 1

FIG. 2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VIDEO CCT.
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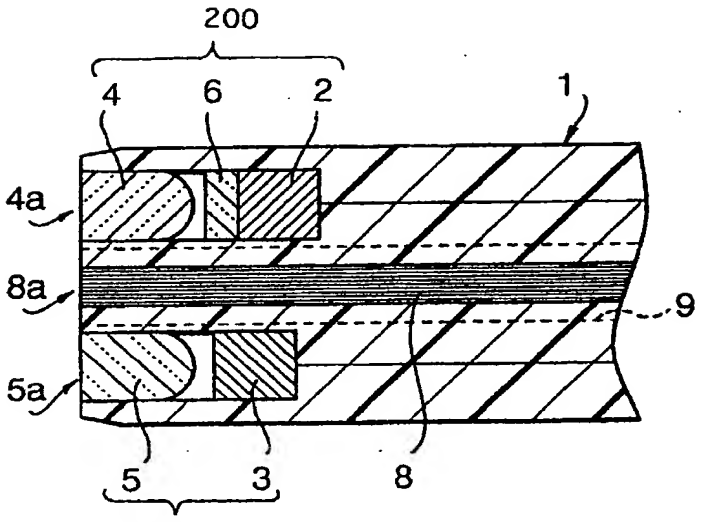

FIG. 3

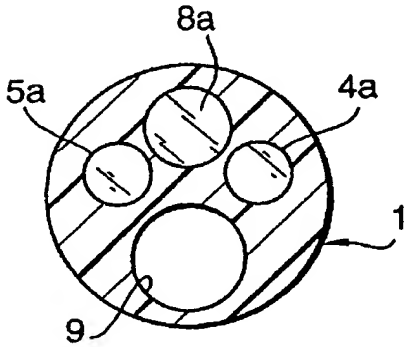

FIG. 4

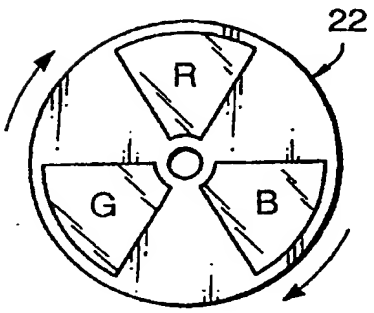

FIG. 5

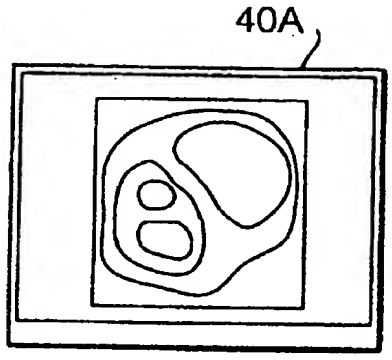

FIG. 6

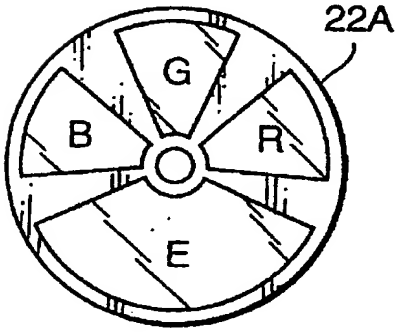

FIG. 7

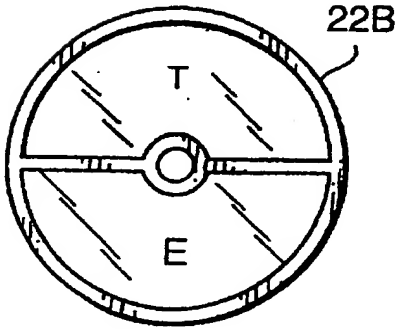

FIG. 8

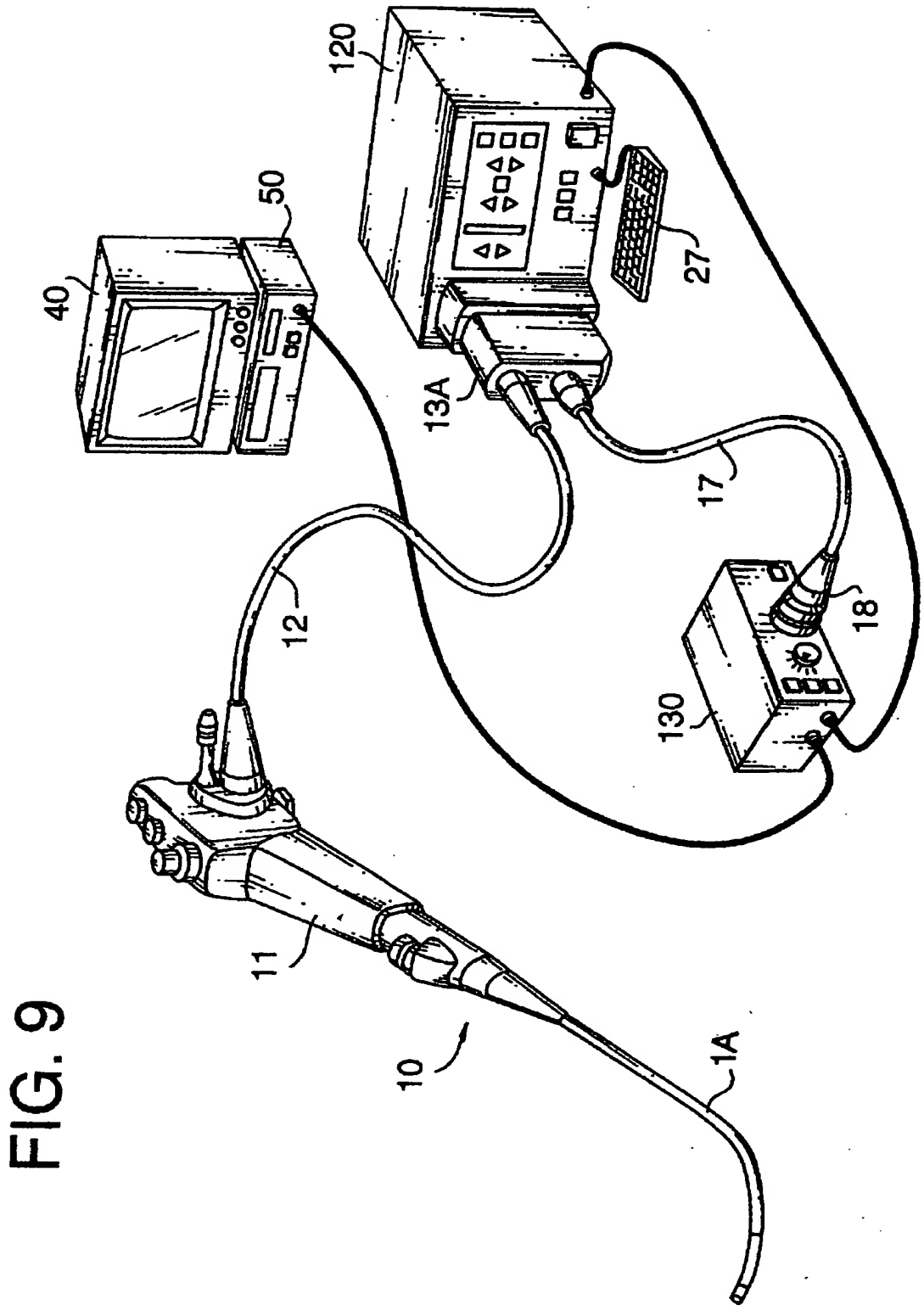

FIG. 9

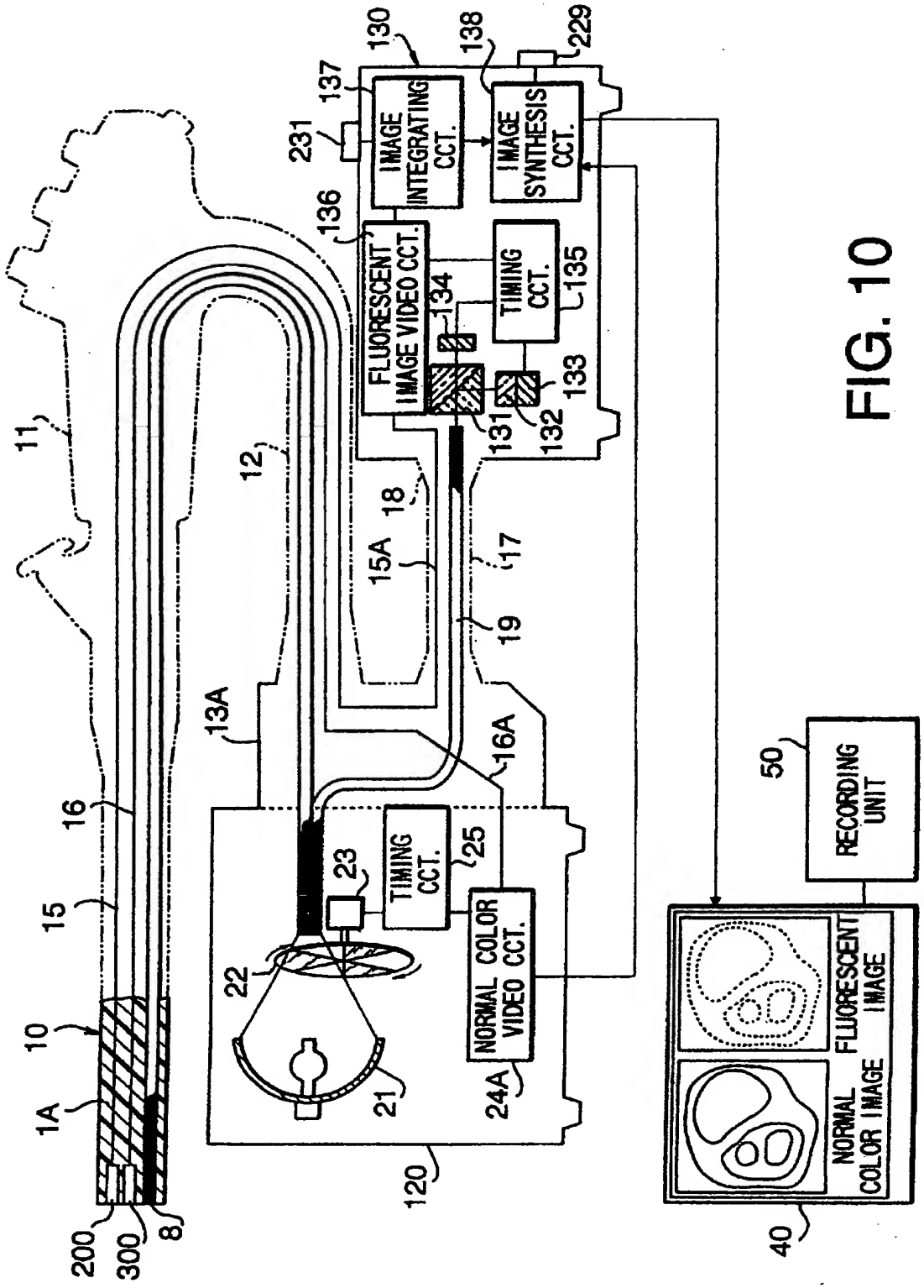
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IMAGE SYNTHESIS CCT.
FLUORESCENT IMAGE VIDEO CCT.
TIMING CCT.
TIMING CCT.
NORMAL COLOR VIDEO CCT.
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FLUORESCENT IMAGE
130
138
229
137
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131
132
11
12
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15A
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FIG. 10

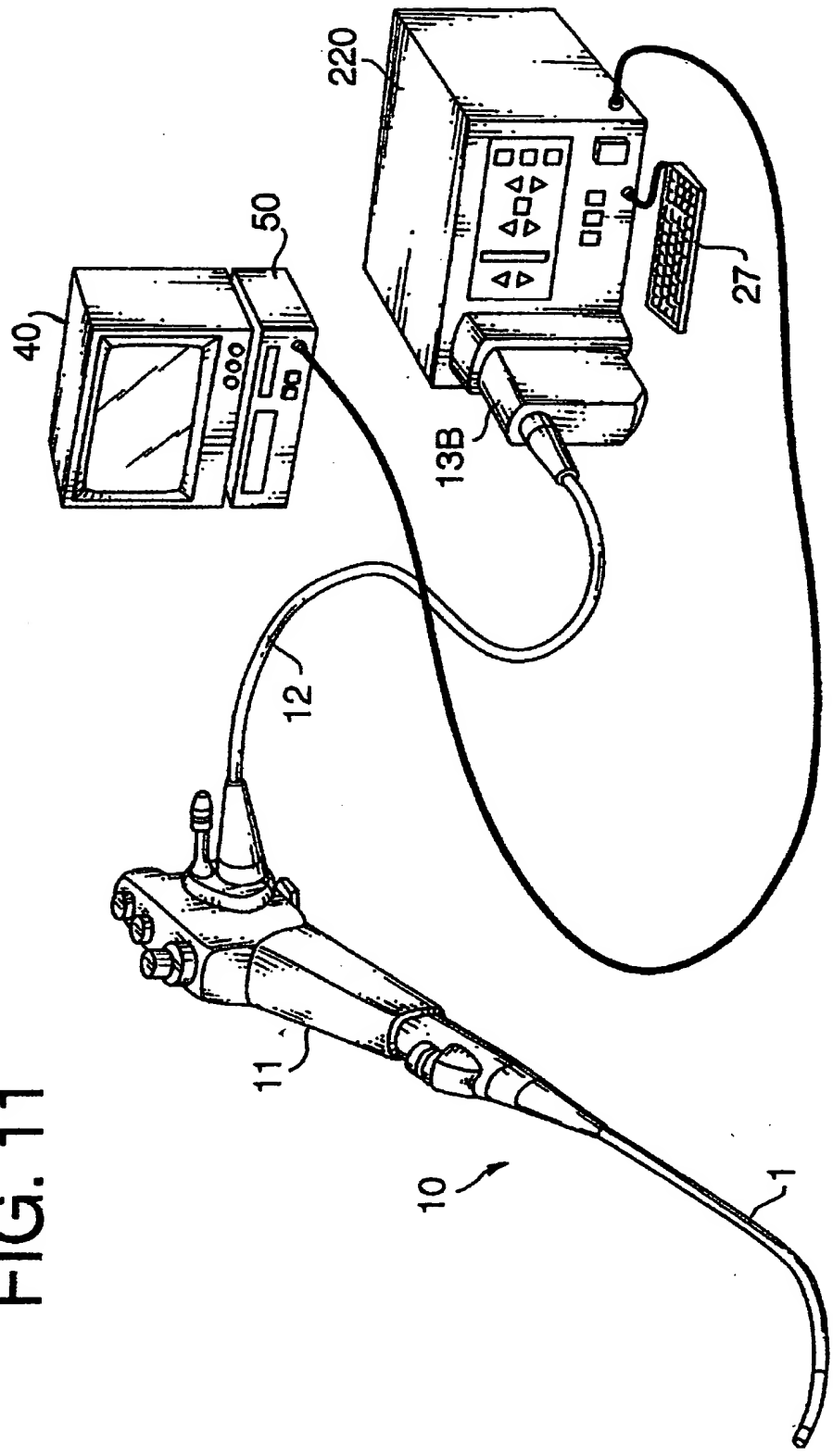
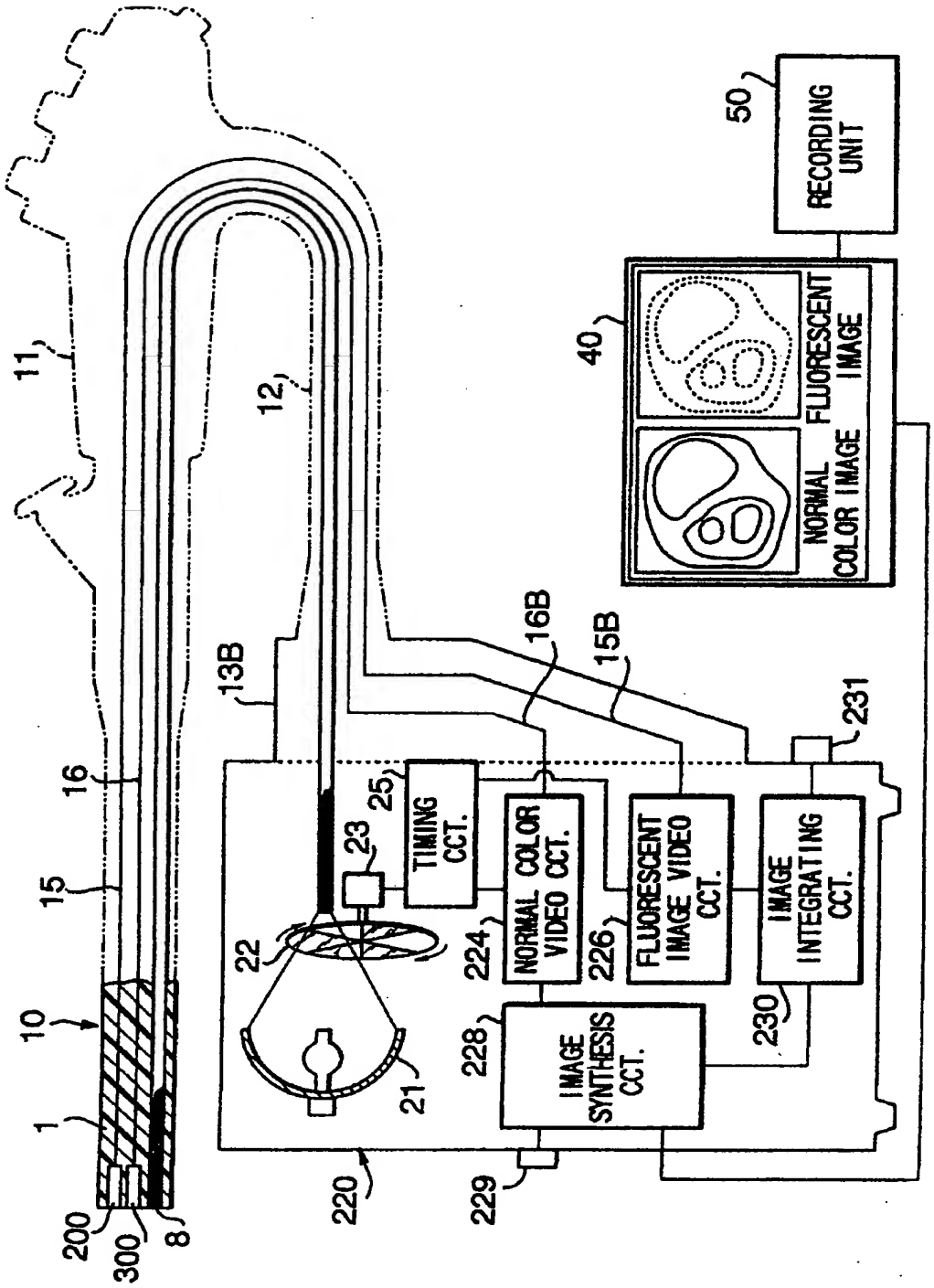


FIG. 11

FIG. 12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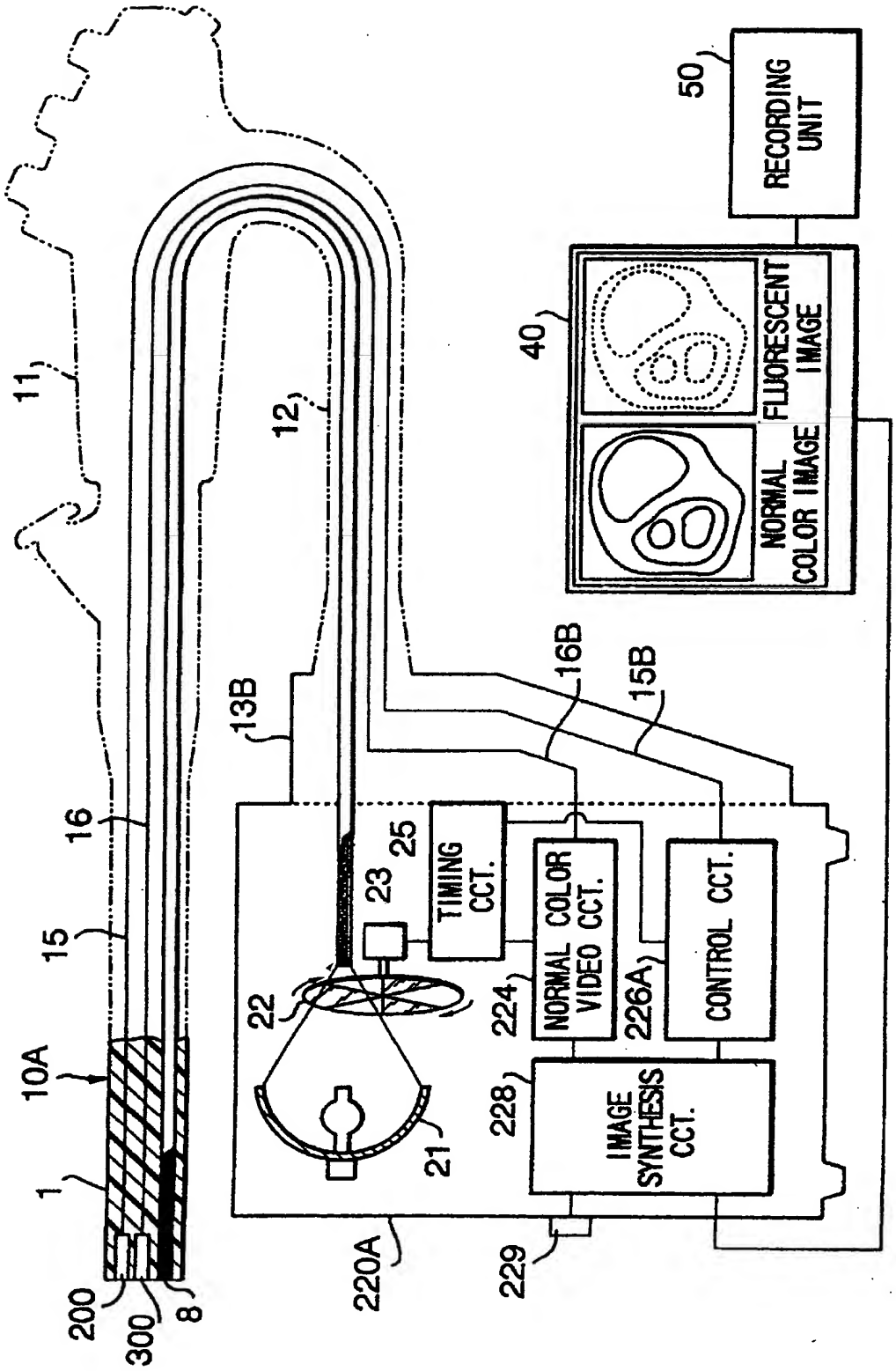
8
220
224
NORMAL COLOR VIDEO CCT.
226
FLUORESCENT IMAGE VIDEO CCT.
IMAGE INTEGRATING CCT.
230
228
IMAGE SYNTHESIS CCT.
229

FIG. 13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CONTROL CCT.
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IMAGE SYNTHESIS CCT.
21
220A
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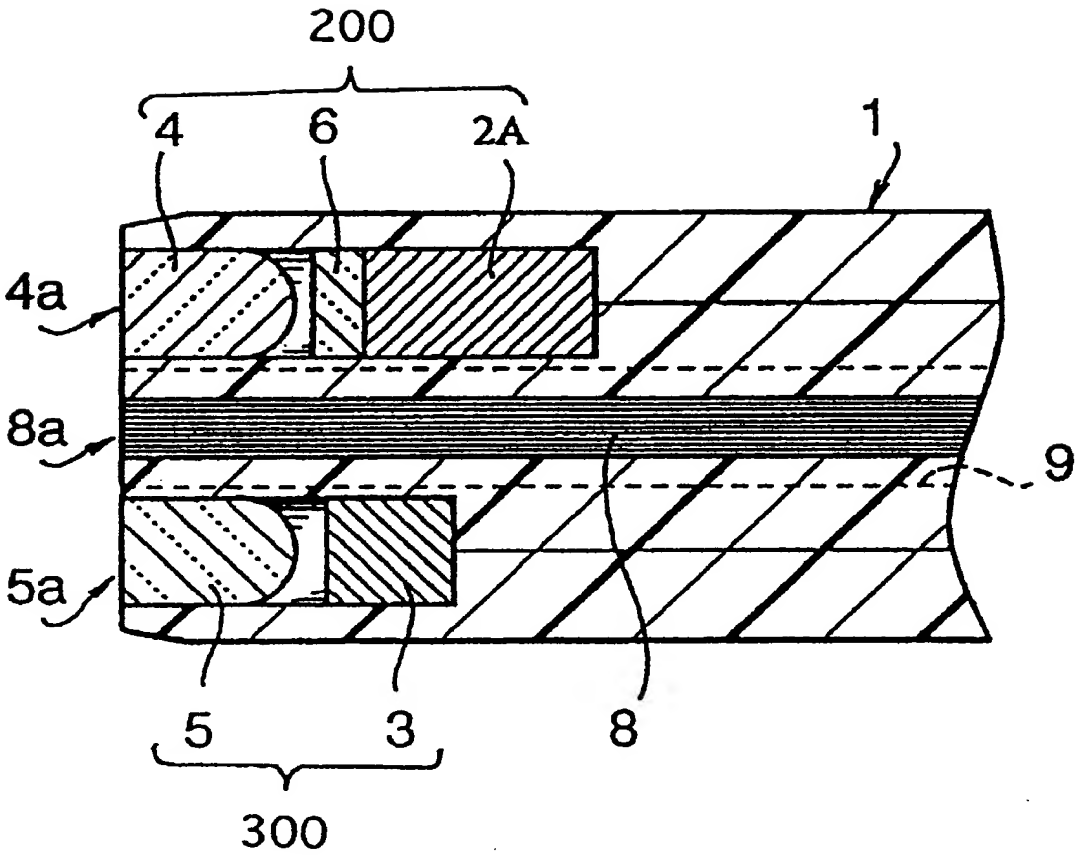
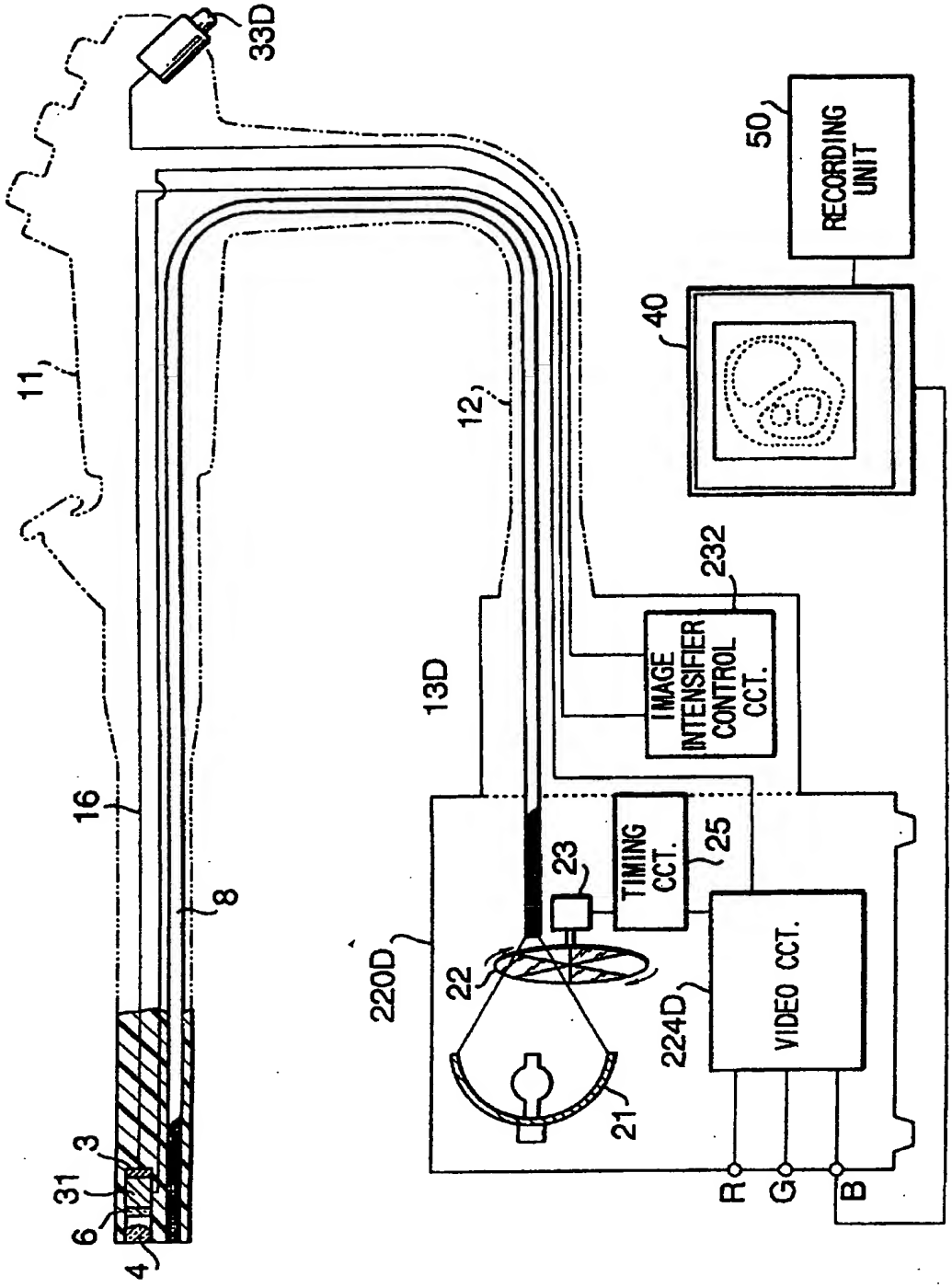


FIG. 14

FIG. 15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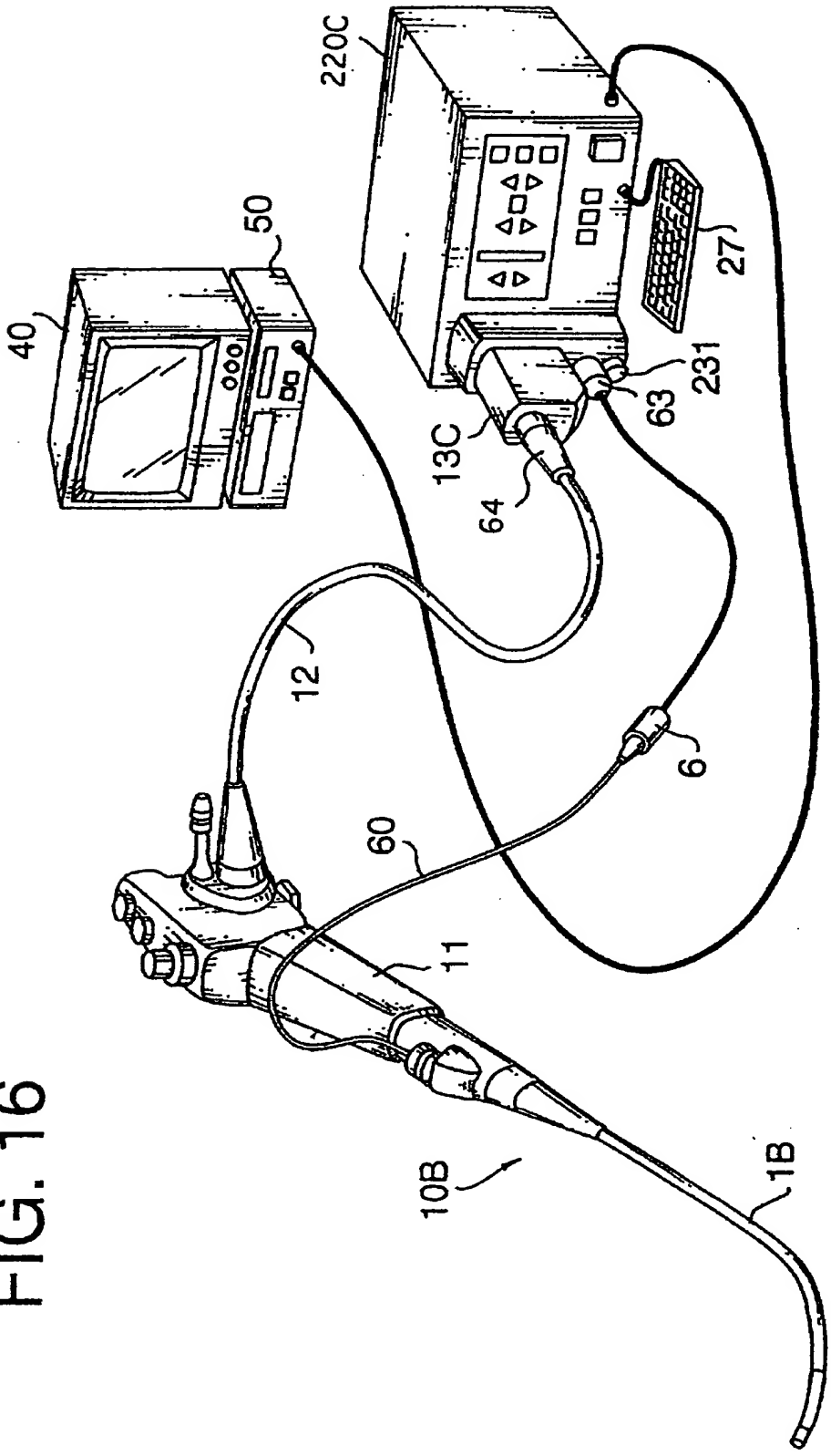
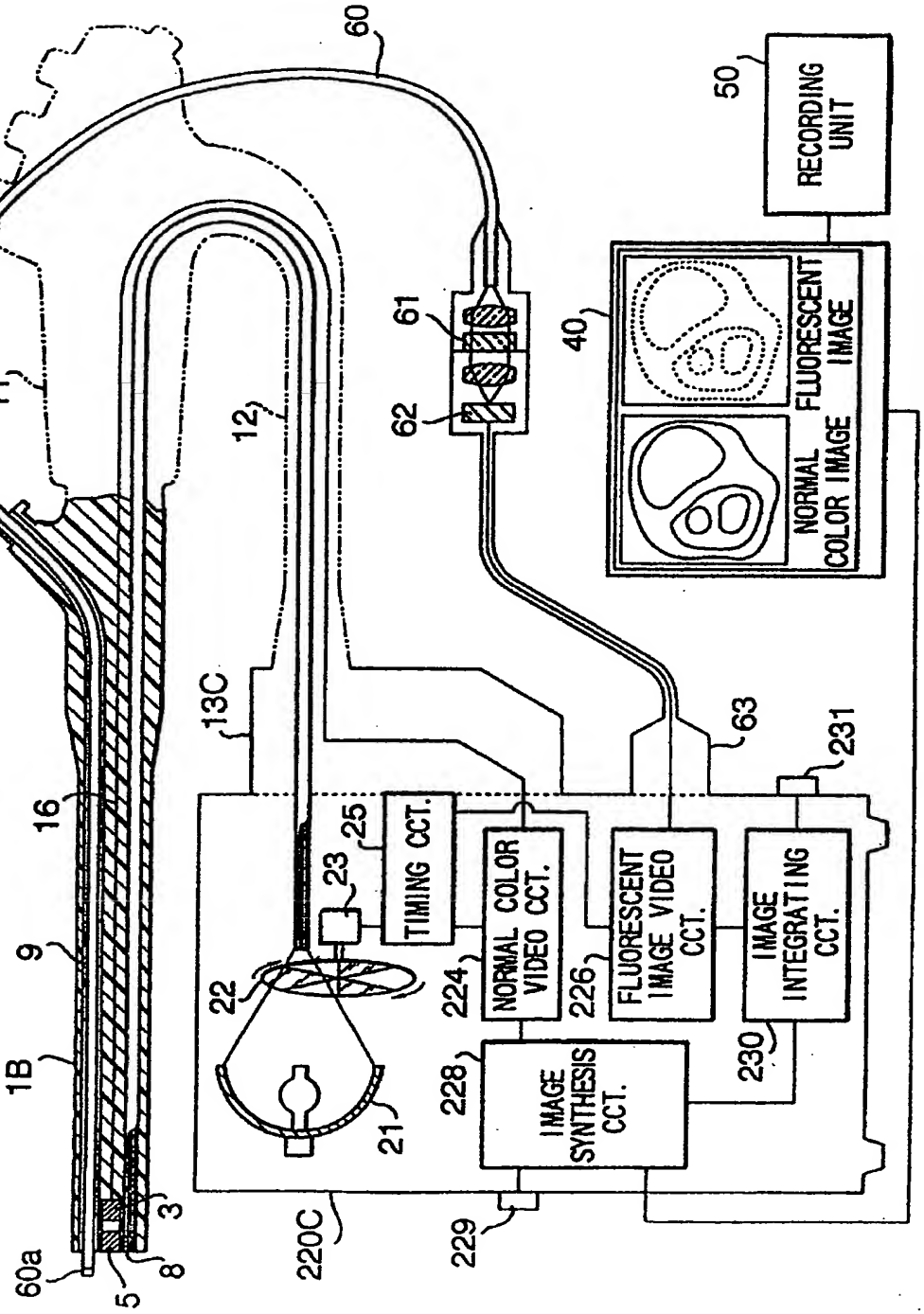


FIG. 16

FIG. 17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TIMING CCT.
23
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224
NORMAL COLOR VIDEO CCT.
226
FLUORESCENT IMAGE VIDEO CCT.
IMAGE INTEGRATING CCT.
1B
228
IMAGE SYNTHESIS CCT.
230
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FLUORESCENCE DIAGNOSIS ENDOSCOPE SYSTEM

This application is a continuation of application Ser. No. 08/531,921, filed Sep. 21, 1995, now abandoned.

BACKGROUND OF THE INVENTION

The invention relates to an endoscope system for observing human tissue that is illuminated with light having a predetermined wavelength. More specifically, the human tissue is illuminated with light having a predetermined wavelength, resulting in the tissue fluorescing at another predetermined wavelength. The fluorescence of the tissue is detected and processed by the endoscope system.

It is known that when human tissue is illuminated by light which has a wavelength of between 400 nm (nanometers) and 480 nm (hereinafter referred to as excitation light), the tissue will fluoresce (glow), thereby producing light having an approximate wavelength between 520 nm and 600 nm. Cancerous human tissue, however, does not fluoresce even if it is illuminated with the excitation light. Therefore, cancer at an early stage, which may not be detected during a normal endoscope observation, can be detected with an endoscope system which illuminates the tissue with the excitation light (i.e., a fluorescence diagnosis endoscope system).

A conventional fluorescence diagnosis endoscope system has an excitation light filter between a light source and a light path of an endoscope, and a fluorescent light filter between an objective optical system and an image receiving element provided at the insertion side of the endoscope. The excitation light filter allows only the excitation light to pass through, and the fluorescent light filter allows only the fluorescent light to pass through. An example of such an endoscope system is disclosed in a Japanese Patent Provisional Publication HEI 4-150845.

Since the intensity of the light produced by the fluorescence of the illuminated tissue is low, it is sometimes difficult to obtain a fluorescent image of the tissue that is sufficiently bright. Therefore, it is difficult to perform the diagnosis accurately.

Further, due to the construction of the conventional fluorescence diagnosis endoscope system described above, only the light produced by the fluorescence of the tissue enters the image receiving element of the endoscope. Thus, the organs or the tissue cannot be observed when the tissue is illuminated with normal light by the same endoscope. In order to examine the organs or tissue using normal light, the endoscope for the fluorescence diagnosis is removed and another endoscope for the normal observation is inserted. This is both time consuming and disruptive during an examination of a person.

SUMMARY OF THE INVENTION

It is therefore a first object of the invention to provide an proved endoscope system which is capable of processing the low intensity of the fluorescent light to obtain a bright image, using the fluorescence diagnosis endoscope system.

Another object of the invention is to provide an endoscope system capable of improving the facility of observation of the person when both the fluorescence diagnosis and the diagnosis with the normal light are performed.

A further object of the invention is to provide an endoscope capable of outputting image signal sufficient to perform diagnosis when the fluorescence image is received.

A still further object of the invention is to provide an endoscope system in which general purpose endoscope is utilized to configure the fluorescence diagnosis system.

A further object of the invention is to provide a video processing unit to which either a general purpose endoscope or an endoscope for the fluorescence diagnosis can be connected.

For the above objects, according to an aspect of the invention, there is provided a fluorescence diagnosis endoscope system used to observe human tissue, the system including a light source for illuminating the human tissue with light having a plurality of wavelength ranges, the human tissue fluorescing in response to illumination with excitation light. The excitation light has a predetermined wavelength range within the plurality of wavelength ranges. The system also includes a pair of image receiving elements, each of the pair of image receiving elements outputting an image signal corresponding to an optical image. An objective optical system for forming optical images of the human tissue is provided on each of the pair of image receiving elements, and a filter for transmitting light produced by the fluoresced tissue is arranged between one of the pair of image receiving elements and the objective optical system. Also included is a device for selecting one of the pair of image receiving elements, a device for processing the image signal output by each one of the pair of image receiving elements, and a device for outputting a video signal output by the selected one of the pair of image receiving elements. With this endoscope system, human tissue can be observed using a normal image and a fluorescence image without exchanging the endoscope. Therefore, the facility of observing the person can be improved.

The predetermined wavelength of the excitation light is preferably in a range between 400 nm and 480 nm. Further, the light emitted by the fluoresced tissue has a wavelength in a range between 500 nm and 600 nm.

The endoscope system can be constructed such that the light source illuminates the object with red, green and blue light, and the blue light is used as the excitation light. Therefore, another light source for emitting the excitation light is not required.

Alteratively, the light source can be constructed such that a lamp and a plurality of filters respectively transmit light having different wavelengths, with one of the plurality of filters transmitting only the excitation light. For example, the system may be provided with a red, green and blue filter as well as the filter for transmitting the excitation light. Generally the transmission wavelength range of the blue filter is 400 nm–500 nm. The excitation light filter can be a filter having the transmission wavelength range of 400 nm–480 nm. According to this range, the fluorescent light filter can be a filter having a wider transmission wavelength range of 480 nm–600 nm.

According to another aspect of the invention, there is provided an endoscope for receiving fluorescent light emitted by an object including an image receiving element for receiving an image of the object and for outputting an image signal corresponding to the received image. The image receiving element includes a device for amplifying the image signal, an objective optical system for forming the image of the object on the image receiving element, and a device for transmitting light having a predetermined wavelength range to the object for illuminating the object. The object producing the fluorescent light has another predetermined wavelength range, in response to illumination with light having the predetermined wavelength range. A filter is provided between the image receiving element and the objective optical system for transmitting light having the another predetermined wavelength range, and for not trans-

mitting light having the predetermined wavelength range. Therefore, according to this system, even if the intensity of the fluorescent light emitted by the human tissue is relatively low, it can be dealt with and a bright fluorescence image can be obtained.

Optionally, the image receiving element comprises an amorphous silicon multi-layer amplified MOS imager.

Alternatively, the image receiving element includes a CCD associated with an image intensifier.

Further, the image intensifier is capable of changing its sensitivity, and the sensitivity is set relatively low when the object emits light having a wavelength range outside the range between 500 nm to 600 nm, while the sensitivity is set relatively high when the object emits the wavelength range between 500 nm to 600 nm. According to a further aspect of the invention, there is provided a fluorescence diagnosis endoscope system including an electronic endoscope for receiving an image of an object to be observed and for outputting an image signal corresponding to the received image. A light source is provided for illuminating the object with light having at least one of a plurality of wavelength ranges. The light source illuminates the object with an excitation light having a predetermined wavelength range, and the object fluoresces in response to illumination by the excitation light. The system also includes a fiber scope for inserting into an instrument channel of the electronic endoscope, the fiber scope having a lens at a tip for converging the light from the object and for transmitting the light through the fiber scope. Also included is an image receiving element for receiving the light transmitted through the fiber scope, and a filter provided between an end of the fiber scope opposite the tip and an other image receiving element, the filter transmits light emitted by the fluoresced object and does not transmit light having the predetermined wavelength range. A device for receiving the image signal output by the image receiving element and outputting a corresponding video signal is also provided.

According to this system, the conventional endoscope only having an image receiving element for the normal image is used, and the fluorescence image can also be observed without exchanging the endoscope.

According to still another aspect of the invention, there is provided an endoscope including a pair of image receiving elements for receiving images and for outputting image signals corresponding to the received images, an objective optical system for forming the images on the image receiving elements, and a device for transmitting light having a predetermined wavelength range to illuminate an object. The object fluoresces in response to illumination with the transmitted light. A filter is provided between one of the image receiving elements and the objective optical system for transmitting light emitted by said fluoresced object and for preventing transmission of light reflected by the object. According to this system, since the endoscope has two image receiving elements for the normal image and for the fluorescence image, and the image receiving elements output respective image signals, the normal image and the fluorescence image can be selectively observed without exchanging the endoscope.

According to a further aspect of the invention, there is provided a video processing unit for an endoscope including:

a first and a second image signal processing circuits, each of the image signal processing circuit being capable of receiving an image signal transmitted from an image receiving element of an endoscope, the first and the second image signal processing circuits having different characteristics. Thus, depending on the condition of light which is incident to an image receiving element of an endoscope, the first and the second image signal processing circuit is selectively used.

Optionally, the first image signal processing circuit can receive image signals representative of a plurality of frames corresponding to a plurality of components of light, and output a frame of a color video signal.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

FIG. **1** shows a fluorescence diagnosis endoscope system as a first embodiment of the invention;

FIG. **2** is a block diagram illustrating the endoscope system of FIG. **1**;

FIG. **3** shows a schematic top view of the insertion side portion of the endoscope used in the endoscope system of FIG. **1**;

FIG. **4** is a front view of the insertion side portion of the endoscope used in the endoscope system of FIG. **1**;

FIG. **5** is a front view of a light filter;

FIG. **6** shows an image seen on a monitor of fluoresced tissue;

FIG. **7** shows a modification of the light filter shown in FIG. **5**;

FIG. **8** shows another modification of the light filter shown in FIG. **5**;

FIG. **9** shows a fluorescence diagnosis endoscope system according to a second embodiment of the present invention;

FIG. **10** is a block diagram illustrating the endoscope system shown in FIG. **9**;

FIG. **11** shows a fluorescence diagnosis endoscope system according to a third embodiment of the present invention;

FIG. **12** is a block diagram illustrating the endoscope system shown in FIG. **11**;

FIG. **13** is a block diagram of a fluorescence diagnosis endoscope system according to a fourth embodiment of the present invention;

FIG. **14** shows a schematic cross section of the insertion side portion of the endoscope used in the endoscope system shown in FIG. **13**;

FIG. **15** is a block diagram of a fluorescence diagnosis endoscope system according to a fifth embodiment of the present invention;

FIG. **16** shows a fluorescence diagnosis endoscope system according to a sixth embodiment of the present invention; and

FIG. **17** is a block diagram illustrating the endoscope system shown in FIG. **16**.

DESCRIPTION OF THE EMBODIMENT

FIG. **1** shows a fluorescence diagnosis endoscope system according to a first embodiment of the invention. FIG. **2** is a block diagram illustrating the endoscope system of FIG. **1**.

As shown in FIG. **1**, the endoscope system is provided with an electronic endoscope **10**, a video processing unit **20**, a switching unit **30**, a display unit **40**, and a recording unit **50**.

The endoscope **10** has a flexible tube **1** which is inserted inside a human body to be observed, and an operation section **11** which controls the operation of the endoscope **10**.

As shown in FIGS. **2** and **3**, at the insertion end portion of the tube **1**, first and second optical units **200** and **300** are

provided. The optical units **200** and **300** respectively include a first image receiving element **2** and a second image receiving element **3**. In the first embodiment, the first and second image receiving elements **2** and **3** are monochromatic CCDs (Charge Coupled Devices). FIG. **2** is not an actual cross-sectional view, but shows the relationship between the optical units **200** and **300** with respect to the entire system. The actual cross-sectional arrangement of the image receiving elements **2** and **3** as well as other elements described below, are shown in FIG. **3**.

In front (on left-hand side of FIG. **2** or FIG. **3**) of the first and second image receiving elements **2** and **3**, a first optical system **4** and a second optical system **5** are provided. The first and second optical systems **4** and **5** form images of an object to be observed on the first and second image receiving elements **2** and **3**, respectively.

A fluorescent light filter **6** is located between the first optical system **4** and the first image receiving element **2**. No such filters are provided between the second objective optical system **5** and the second image receiving element **3**. The fluorescent light filter **6** has a transmission wavelength range greater than the wavelength of the excitation light, thereby preventing the excitation light from being transmitted therethrough. In the following embodiments, the transmission wavelength of the fluorescent light filter **6** is between 520 nm (nanometers) and 600 nm.

A light guide fiber bundle **8** is provided in the endoscope **10**. The light emitting surface of the light guide fiber bundle **8** is arranged adjacent to the first and second optical systems **4** and **5**, and emits light towards the object (i.e., tissue) to be observed.

FIG. **4** is a front view of the insertion end of the tube **1**. Light receiving windows **4***a* and **5***a* are formed on the incident surface of the insertion end of the tube **1**, in front of the first and second optical systems **4** and **5**, respectively. A light emitting window **8***a* is also formed on the incident surface of the insertion end of the tube **1**, in front of the light emitting end of the light guide fiber bundle **8**. A channel **9** is formed through the tube **1**, where forceps or operational instruments can be inserted.

As shown in FIG. **1**, the tube **1** is connected to the operation section **11**. The operation section **11** is connected to the video processing unit **20** through a flexible cable **12** and a connector **13**.

A first cable **15**, a second cable **16** and the light guide fiber bundle **8** are inserted through the tube **1**, the operation section **11**, and the flexible cable **12**. The first and second cables **15** and **16** transmit signals between the video processing unit **20**, and the first and second image receiving elements **2** and **3**.

As shown in FIG. **2**, a light source **21**, which includes a Xenon lamp and a reflector, is provided in the video processing unit **20**. Light emitted by the light source **21** enters a light receiving surface of the light guide fiber bundle **8**. A disk-shaped RGB (Red, Green and Blue) filter **22** is provided in the light path between the light source **21** and the light receiving surface of the light guide fiber bundle **8**.

FIG. **5** shows a front view of the RGB filter **22**. The RGB filter **22** is constructed such that red, green and blue filter sectors are arranged about an axis of rotation of the filter **22** with light shielding portions provided between the filter sectors. The transmission wavelength ranges for the red, green and blue filter sectors employed in the embodiments described below, are as follows: Each filter sector has a transmissivity of greater than 50 percent for light having a wavelength within the transmission wavelength range. The red filter sector has the transmission range of 580–650 nm; the green filter sector has the transmission range of 500–580 nm; and the blue filter sector has the transmission range of 400–500 nm. The RGB filter **22** is rotated at a constant speed by a motor **23**. As the RGB filter **22** rotates, the object to be observed, which is located in front of (i.e., on the left-hand side of FIG. **2**) the insertion end of the tube **1**, is illuminated by red, green and blue light, sequentially and periodically.

The first and second cables **15** and **16** are connected to a switching circuit **26** provided in the connector **13**. The switching circuit **26** is connected with a manually operable selector **28**. The selector **28** connects one of the cables **15** and **16** to a video circuit **24** in order to process a normal image signal. A foot switch can also be used as the selector **28**.

The first and second image receiving elements **2** and **3**, and the motor **23** are driven synchronously with each other in accordance with an output signal of a timing circuit **25**. Thus, an image of the object is detected using an RGB frame-sequential method.

The video circuit **24** receives the image signals transmitted from the light receiving elements **2** and/or **3**, and outputs RGB color video signals in order to display a color image of the object to be observed, on the screen of the display unit **40**.

In the first embodiment, the RGB video signals are transmitted to the display unit **40** through the switching unit **30**. The switching unit **30** has two modes of operation. In the first mode of operation, all the RGB signals are transmitted to the display unit **40**. In the second mode of operation, the B (blue) signal (when the object is illuminated with the blue light) is transmitted to the display unit **40**. The first mode of operation allows the normal color image to be displayed, while the second mode of operation allows the fluorescence image of the object to be displayed on the screen of the display unit **40**.

The display unit **40** is connected with the recording unit **50** which records the video signal onto a magnetic recording medium, such as a video tape. The video processor **20** is connected to a keyboard **27** for inputting various operational commands.

In the endoscope system described above, the R, G, and B components of the image signals are output by the first and second image receiving elements **2** and **3**, as the RGB filter **22** rotates. Therefore, by connecting the second cable **16** to the video circuit **24** through the switching circuit **26**, a normal color video signal is output from the video circuit **24**. Therefore, in the first mode of operation of the switching unit **30**, all the RGB signals are transmitted to the display unit **40**, and the normal color image is displayed on the screen.

The first light receiving element **2** outputs an image signal corresponding to the light having a wavelength approximately between 520 nm and 600 nm, since only the light which passed through the fluorescent light filter **6** is incident on the first light receiving element **2**.

When the switching circuit **26** connects the first signal cable **15** to the video circuit **24**, and controls the switching unit **30** to operate in the second mode of operation, the image signal when the object is illuminated with blue light (having wavelength between 400 nm and 500 nm) is selected. Therefore, the video signal representative of the fluorescent image is transmitted to the display unit **40**. Thus, the fluorescent light image **40A** is displayed on the screen of the display unit **40**, as shown in FIG. **6**. In the embodiment, the actual color of the fluorescent light image is green. However,

according to the configuration of the system, a blue image is displayed which represents the fluorescent light image.

As described above, according to the first embodiment, both the normal color image and the fluorescence image can be observed with a single endoscope. There is thus no need to change the endoscopes and the operation of the endoscope between operational modes is neither time consuming nor disruptive to the examination of the person. This facilitates, e.g., the early diagnosis of cancer in the body.

Since the system can selectably transmit the RGB video signal, or the fluorescence image video signal to the display unit **40**, an off-the-shelf video processing device can be used for the video processing unit **40**.

In the first embodiment, the RGB filter **22** has three sectors (filters) which transmit red, green and blue components, respectively, of light when the normal color image is observed. Further, the light transmitted through the blue filter (the blue light) is also used as the excitation light when the fluorescent image is observed.

In a modification to the first embodiment, a modified filter **22A**, shown in FIG. **7**, is employed. The modified filter **22A** has RGB filters and another filter E. Depending on the portion of the human tissue to be observed, the fluorescent light way have a wavelength range between 480 and 500 nm. This range of the fluorescent light overlaps the upper side of the transmission wavelength range of the blue filter. In order to obtain only a fluorescent image of the tissue (and not a normal image), the filter E should have an upper limit of the transmission wavelength range which is lower than the upper limit of the transmission wavelength range of the blue filter (i.e., 500 nm), and lower than 480 nm. The filter E transmits the excitation light having the wavelength between 400 nm and 480 nm. When this modified filter **22A** is used, the filter E transmits the excitation light to the object to be observed. When the filter E is used, a filter having the transmission wavelength range of 480–600 nm is used as the fluorescent light filter **6**.

In a second modification of the first embodiment, the filter **22** is replaced with a modified filter **22B**, shown in FIG. **8**. Further, in the second modification of the first embodiment, a color image receiving element is used instead of the monochromatic image receiving element **3**. The modified filter **22B** does not have RGB filters since the color image receiving element does not require such a filter. The modified filter **22B** has a transparent filter T (this could also be an opening) and the filter E, for transmitting the excitation light.

FIG. **9** shows a fluorescence diagnosis endoscope system according to a second embodiment of the invention, and FIG. **10** is a block diagram illustrating the endoscope system of FIG. **9**. In the description, the devices, which are similar to those of the first embodiment have the same reference numerals, and will not be described below.

As shown in FIG. **9**, the endoscope system according to the second embodiment includes the endoscope **10**, a normal video processing unit **120**, a fluorescent light image control unit **130**, the display unit **40** and the recording unit **50**.

The endoscope **10** is connected to the video processing unit **120** through the flexible cable **12** and a connector **13A**. The fluorescent light image control unit **130** is connected to the video processing unit **120** through a connector **18**, a flexible cable **17** and the connector **13A**.

The video processing unit **120** has the light source **21**, the filter **22**, the motor **23**, and the timing circuit **25** which are similar to those in the video processor **20** of the first embodiment. The video processing unit **120** also includes a video circuit **24A** which processes only the image signal output by the second image receiving element **3**.

The second image receiving element **3** is connected to the video circuit **24A** through the second cable **16**, and a cable **16A** provided in the connector **13A**. The second image receiving element **3** is driven synchronously with the rotation of the motor **23** in accordance with the timing signal output by the timing circuit **25**.

The first image receiving element **2** is connected to a video circuit **136** through the cable **15** and a cable **15A**, which is provided in the flexible cable **17**.

A light guide **19** is provided in the flexible cable **17**. The light incident surface of the light guide **19** is arranged adjacent to the light incident surface of the light guide fiber bundle **8**. The light emitting side of the light guide **19** is provided inside the connector **18**.

In the fluorescence image control unit **130**, a beam Splitter **131** is provided such that a light receiving surface thereof contacts the light emitting surface of the light guide **19**. Light emitted from the light emitting surface of the light guide **19** is split by the beam splitter **131** into two rays of light. One ray is incident on a first light receiving device **133** through a blue filter **132**, and the other ray is incident on a second light receiving device **134**. Output signals of the first and second light receiving devices **133** and **134** are transmitted to a timing circuit **135**. Thus, the timing circuit **135** and the timing circuit **25** generate the timing signals synchronously with each other.

As described above, the first signal cable **15** is connected to the video circuit **136**. As the timing signal is transmitted from the timing circuit **135**, the video circuit **136** detects the image signal only when the object is illuminated with a blue light having wavelength between 400 nm and 475 nm. Since the image signal received by the video circuit **136** corresponds to light transmitted through the filter **6**, the image is the fluorescence image.

In an image integrating circuit **137**, a plurality of frames of the fluorescent light image output by the video circuit **136** is integrated (superimposed) to form a single bright image. Then, the bright image signal is sent to a image synthesis circuit **138**. In the second embodiment, the number of frames to be superimposed can be set either manually by means of a switch **231**, or automatically.

The image synthesis circuit **138** receives the integrated fluorescent light video signal from the image integrating circuit **137**, and the color video signal from the normal video circuit **24A**. Then, in accordance with the operated status of a display switch **229**, the image synthesis circuit **138** outputs one of the normal color video signal or fluorescence image video signal, or both to the display unit **40**.

According to the second embodiment, the first image receiving element **2** is connected to a video processor which is provided separately from the normal video processing unit **120**. Further, an off-the-shelf video processor can be used for processing the image observed using the normal color video signal.

FIG. **11** shows a fluorescence diagnosis endoscope system according to a third embodiment of the present invention. FIG. **12** is a block diagram illustrating the endoscope system of FIG. **11**.

The system of the third embodiment consists of the endoscope **10**, a video processing unit **220**, the display unit **40** and the recording unit **50**. The endoscope **10** and the video processing unit **220** are connected by the flexible cable **12** through a connector **13B**. The keyboard **27** for inputting commands etc. is connected to the video processing unit **220**.

The light source **21**, the filter **22**, the motor **23** and the timing circuit **25** are provided in the video processing unit **220**. Further, the video processing unit **220** is provided with a video circuit **224** for processing a normal color image, a video circuit **226** for processing the fluorescent light image, an image integrating circuit **230**, and an image synthesizing circuit **228**. The video circuits **224** and **226**, the image integrating circuit **230**, and the image synthesizing circuit **228** function in a similar way to the video circuits **24A** and **136**, the image integrating circuit **137**, the image synthesizing circuit **138**, respectively of the second embodiment. Therefore, these circuits will not be described in detail.

The first image receiving element **2** is connected to the video circuit **226** through the cable **15** and a cable **15B**, which is provided in the connector **13B**. The second image receiving element **3** is connected to the video circuit **224** through the cable **16** and a cable **16B**, which is provided inside the connector **13B**. In accordance with the output signal of the timing circuit **25**, the video circuit **226** outputs only the video signal corresponding to the fluorescent light image.

In the third embodiment, the video signal output by the video circuit **226** is integrated by the integrating circuit **230**, in a similar manner described for the second embodiment above. The number of frames to be superimposed is set by the manually operable switch **231**. By operating a screen switch **229**, the image to be displayed on the screen of the display unit **40** can be selected.

In the third embodiment, based on the output signal of the timing circuit **25**, the first and second image receiving elements **2** and **3** are driven synchronously with the rotation of the motor **223**, and accordingly the RGB frame-sequential image reading is performed.

The video circuit **226** detects the image signal when the object is illuminated with the blue light (having wavelength between 400 nm and 500 nm). Since the filter **6** is provided in front of the image receiving element **2**, a fluorescence image video signal is output by the video circuit **226**.

The signal output by the video circuit **226** is transmitted to the integrating circuit **230**, and a plurality of frames are integrated. The number of frames to be superimposed by the integrating circuit **230** is set automatically, or by the switch **231**.

FIG. **13** is a block diagram of a fourth embodiment of a fluorescence diagnosis endoscope system according to the present invention. FIG. **14** shows a schematic cross-section of the insertion end of the tube **1** of the endoscope used in the endoscope system shown in FIG. **13**.

The fourth embodiment consists of an endoscope **10A**, a video processing unit **220A**, the display unit **40** and the recording unit **50**.

The fourth embodiment will be described hereinafter in relation to the third embodiment. In the fourth embodiment, instead of the image receiving element **2** of the foregoing embodiments, a very high sensitivity image receiving element **2A** is used in the endoscope **10A**. Further, the video circuit **226** and the integrating circuit **230** are replaced with a control circuit **226A** for controlling the image receiving element **2A**. In the embodiment, the image receiving element **2A** transfers electric charge to an amorphous silicon multi-layered amplified MOS imager to amplify the charge by a factor of over one thousand.

The image receiving element **3** is connected to the video circuit **224** through the cable **16** and a cable **16B** provided inside the connector **13B** to process the normal color image. The image receiving element **2A** is connected to the control circuit **226A**. The video circuit **224** and the controller **226A** are supplied with timing signals from the timing circuit **25**, and drive the image receiving element **3** and **2A**, respectively, and synchronously with the rotation of the filter **22**.

As described above, since the image receiving element **2A** has a very high sensitivity, the integrating circuit is not necessary. The circuitry in the fourth embodiment is therefore simplified. Further, the image output to the monitor **40** is sufficiently bright.

FIG. **15** is a block diagram of a fifth embodiment of a fluorescence diagnosis endoscope system according to the present invention.

In the fifth embodiment, an image intensifier **31** is provided in front of (i.e., at the left-hand side of FIG. **15**) a conventional image receiving element **3**, as shown in FIG. **15**. An image intensifier control circuit **232** for controlling the image intensifier **31** is provided inside a connector **13D**, and a sensitivity adjusting switch **33D** is provided on the operation section **11**.

In the fifth embodiment, by changing the sensitivity of the image intensifier **31**, the brightness of the image is adjusted. Further, since the signal having the adjusted amplitude is input to the video processing unit **220D**, only one video circuit **224D** is required, and the construction of the video circuit **224D** is simple. Further, similar to the previous embodiments, the video circuit **224D** and the image receiving element **3** are driven synchronously with the rotation of the motor **23**, in accordance with the timing signal output by the timing circuit **25**. FIG. **16** shows a fluorescence diagnosis endoscope system according to a sixth embodiment of the present invention. FIG. **17** is a block diagram illustrating the endoscope system shown in FIG. **16**.

The sixth embodiment employs the endoscope **10B**, a video processing unit **220C**, the display unit **40** and the recording unit **50**. Further, the system is provided with a fiber scope **60** which is made of an image guide fiber bundle inserted in the forceps channel **9** of the endoscope **10B**. An objective lens **60*a*** is provided at an insertion end of the fiber scope **60**. The other end of the fiber scope **60** is coupled to a connector **64**. A filter for passing the fluorescent light having a wavelength between 500 nm and 600 nm is provided in the connector **64**. Light emitted by the other end of the fiber scope **60** passes through the filter **61** and is incident on a monochromatic image receiving element **62**. The image receiving element **62** receives a fluorescent image and transmits an image signal to a video circuit **226** through a connector **63**. The function of each element of the video processing unit **220C** is the same as the elements described for the video processing unit **220** of the third embodiment, shown in FIG. **12**.

The present disclosure relates to subject matters contained in Japanese Patent Applications Nos. HEI 6-226521, HEI 6-226522, HEI 6-226523, HEI 6-226524, filed on Sep. 21, 1994, which are expressly incorporated herein by reference in their entireties.

What is claimed is:

1. A fluorescence diagnosis endoscope system for observing living tissue, said system comprising:

a single light source for illuminating the living tissue, said single light source emitting light having a plurality of wavelength ranges, said light comprising visible light and an excitation light;

a filter unit, arranged between said single light source and the living tissue, that periodically filters said light from said single light source, said filter unit comprising at

least one blue, at least one red, and at least one green filters, said blue filters blocking light that does not have a wavelength between 400–500 nm, such that said at least one blue filter is the sole source of light that causes the living tissue to fluoresce and emit fluorescent light;

a first optical unit at the tip of an electronic endoscope, said first optical unit comprising a first optical system and a first imaging element for receiving light reflected from the living tissue by light passing through said green, blue, and red filters to illuminate the living tissue and generating first image signals;

a second optical unit provided at the tip of the electronic endoscope for receiving said fluorescent light and generating second image signals, said second optical unit comprising a second imaging element, a second optical system, and a fluorescence filter provided in front of said second imaging element, the fluorescence filter preventing transmission of light having the same wavelength as the excitation light;

an image processor for processing said first image signals and said second image signals, in accordance with the periodical filtering of light by said filter unit, to form a color image of the living tissue and a fluorescence image of the living tissue, respectively; and

an output device for outputting said color image and said fluorescence image.

2. The fluorescence diagnosis endoscope system according to claim **1**, said output device comprising a selector for selecting at least one of said color image and said fluorescence image for output.

3. The fluorescence diagnosis endoscope system according to claim **2**, further comprising a display for displaying said at least one of said color image and said fluorescence image.

4. The fluorescence diagnosis endoscope system according to claim **2**, said image processor comprising:

a color image processor for forming said color image; and

a fluorescent image processor and an image integrating circuit for forming said fluorescence image, said image integrating circuit integrating a predetermined number of images output by said fluorescent image processor to form said fluorescence image; and

an image synthesizer, responsive to said selector, for outputting at least one of said color image and said fluorescence image.

5. The fluorescence diagnosis endoscope system according to claim **2**, wherein said second optical unit comprises an image amplifier for amplifying said second image signals prior to output, said image processor comprising:

a color image processor for forming said color image;

a control circuit for forming said fluorescence image based on said second image signals; and

an image synthesizer, responsive to said selector, for outputting at least one of said color image and said fluorescence image.

6. A florescence diagnosis endoscope system for observing living tissue comprising:

a single light source for illuminating the living tissue with at least an excitation light that causes the living tissue to fluoresce and emit fluorescent light, said single light source emitting light having a plurality of wavelength ranges, said light comprising visible light and said excitation light;

a filter unit, arranged between said single light source and the living tissue, that periodically filters said light from said single light source, said filter unit comprising at least one red, at least one blue, and at least one green filters that pass light that illuminates the living tissue to obtain an optical image, and said blue filter preventing transmission of light not between 400–500 nm such that said at least one blue filter is the sole source of light that causes the living tissue to fluoresce and emit fluorescent light;

an image receiving element provided at the tip of an electronic endoscope for generating amplified image signals based on a received image of the living tissue;

an optical system for forming said image of the living tissue on said image receiving element;

a fluorescence filter, provided at the tip of the electronic endoscope, and between said optical system and said image receiving element, that transmits said fluorescent light to said image receiving element only when said blue filter is inserted between said single light source and the living tissue, the fluorescence filter preventing transmission of light having the same wavelength as the excitation light; and

a control circuit for controlling and driving said image receiving element to output said amplified image signals in accordance with the periodical filtering of light by said filter unit.

7. The florescence diagnosis endoscope system according to claim **6**, said image receiving element comprising an amorphous silicon multi-layer amplified MOS imager.

8. The florescence diagnosis endoscope system according to claim **6**, said image receiving element comprising a CCD and an image intensifier.

9. The endoscope system of claim **8**, further comprising a signal output unit for outputting video signals corresponding to the color image of the living tissue and the fluorescence image of the living tissue, and a display unit for displaying the color image of the living tissue or the fluorescence image of the living tissue based on the video signals provided as output from the signal output unit.

10. A fluorescence diagnosis endoscope system for observing living tissue comprising:

a single light source;

a light transmitting member, which transmits said light emitted by said single light source toward the living tissue;

a plurality of filters sequentially inserted in an optical path between said single light source and said light transmitting member to filter said light emitted by said single light source, said plurality of filters comprising at least one red, at least one blue, and at least one green filter, said red filters preventing transmission of light not having a wavelength between 580–650 nm, said blue filter preventing transmission of light not having a wavelength between 400–500 nm, and said green filter preventing transmission of light not having a wavelength between 500–580 nm;

said at least one blue filter being the sole source of light that causes the living tissue to fluoresce and emit fluorescent light;

a fluorescent filter;

a first imaging element that receives light reflected from said living tissue; and

a second imaging element receiving fluorescent light emitted from said living tissue and passing through said fluorescent filter.

11. The endoscope system of claim **10**, wherein said first imaging element is mounted at the at the tip of an electronic endoscope.

12. The endoscope system of claim 10, wherein said second imaging element is mounted at the at the tip of an electronic endoscope.

13. The endoscope system of claim 10, further comprising:

said first imaging element emitting a color image signal;

said second imaging element emitting a fluorescent image signal;

a selector for selecting one of said color and fluorescent image signals; and

an image processor, responsive to said selector, for processing said color image signal and said fluorescent image signal in accordance with the sequential filtering by said plurality of filters to form a color image of the living tissue and a fluorescence image of the living tissue, respectively.

14. The endoscope system of claim 10, further comprising a rotating disk upon which said red, blue and green filters are mounted.

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US006280378B1

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Kazuhiro et al.

(10) **Patent No.: US 6,280,378 B1**
(45) **Date of Patent: Aug. 28, 2001**

(54) **FLUORESCENCE ENDOSCOPE**

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(52) **U.S. Cl.** **600/160**; 600/109; 348/65; 348/68

(58) **Field of Search** 600/109, 160; 348/65, 68, 70, 71, 222

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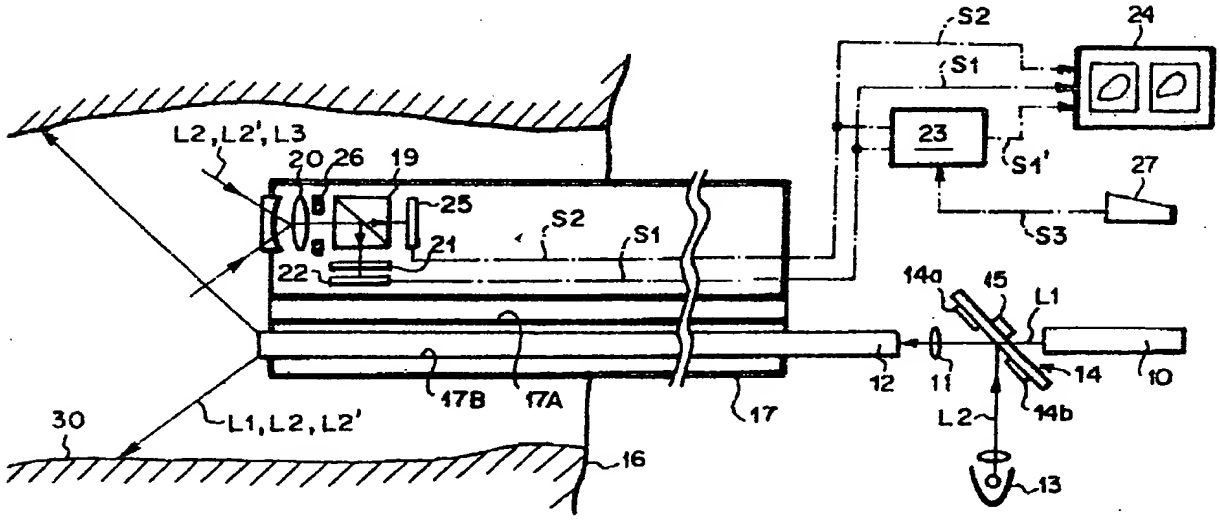
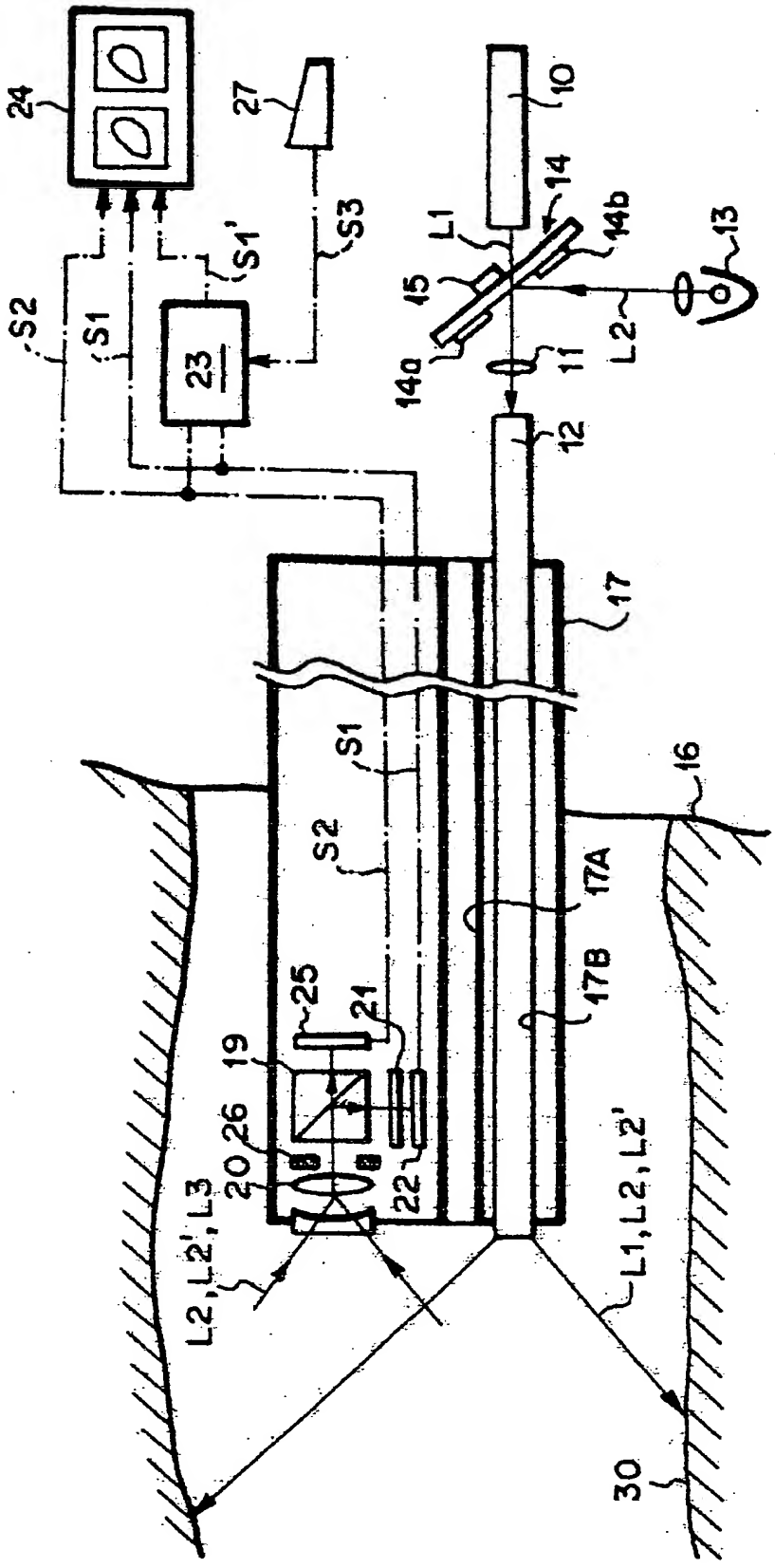
(57) **ABSTRACT**

A fluorescence endoscope includes an illuminating light projecting system which projects illuminating light onto a part inside a body, a first objective optical system which causes the illuminating light reflected at the part of the body to form a normal image of the part, an excitation light projecting system which projects onto a part inside the body excitation light, and a second objective optical system which causes fluorescence emitted from the part to form a fluorescence image. A normal image CCD takes the image formed by the objective optical system and outputs a normal image signal representing the normal image and a fluorescence image CCD takes the fluorescence image formed by the second objective optical system and outputs a fluorescence image signal representing the fluorescence image. A measuring light projecting system projects measuring light at a wavelength in the wavelength range of the fluorescence onto a part inside the body. Measurement images are formed through the first and second objective optical systems by the measuring light reflected at the same part of the body and an image degradation function of the second objective optical system is obtained on the basis of the image signals. An image restoration processing is carried out on a fluorescence image signal on the basis of the image degradation function.

5 Claims, 1 Drawing Sheet




24
27
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L1
14b
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S1'
S3
S2
S1
23
14a
11
L2
12
17
S1
16
S2
17A
17B
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21
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26
20
22
L2,L2',L3
L1,L2,L2'
30

FIG. 1

FLUORESCENCE ENDOSCOPE

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a fluorescence endoscope used to examine the interior of a body cavity and the like, and more particularly to a fluorescence endoscope in which blur of the image due to insufficient depth of focus of the objective optical system can be avoided.

2. Description of the Related Art

There has been in wide use an endoscope to observe the interior of a body cavity or to give treatment observing the interior of a body cavity. Currently an electronic endoscope comprising an illuminating light projecting system which projects illuminating light onto a part inside a body through optical fibers or like, an objective optical system which is inserted into the interior of the body and forms an image of the part by the light reflected at the part of the body and an image taking means which takes the image formed by the objective optical system is major.

On the other hand, there have been made various investigations on photodynamic diagnosis (PDD). The photodynamic diagnosis is a technique in which a photosensitive material which has affinity to tumor and emits fluorescence when excited by light is first administered to the tumor, excitation light having a wavelength in the excitation wavelength range of the photosensitive material is projected onto the tissue, and then the intensity of the fluorescence is measured or the tumor is diagnosed on the basis of an image formed by the fluorescence. As another form of the photodynamic diagnosis, there has been known a technique in which excitation light having a wavelength in the exciting range of a photosensitive material inherent to the organism is projected onto the organism to cause the intrinsic photosensitive material to emit fluorescence (so-called auto-fluorescence), and tumor is diagnosed on the basis of an auto-fluorescence image.

A fluorescence endoscope for taking such a fluorescence image and displaying the image basically comprises an excitation light projecting system which projects excitation light onto a part inside the body in addition to said illuminating light projecting system, the objective optical system and the image taking means, and an image formed by fluorescence emitted from the part is taken by the image taking means.

In such a fluorescence endoscope, use of an objective optical system which is small in F-number and high in numerical aperture is generally required in order to efficiently detect fluorescence which is normally very weak. However when a high numerical aperture objective optical system is used, the depth of focus is reduced and blur is apt to be generated in a part of the fluorescence image taken.

As an objective optical system for an endoscope in which the depth of focus can be increased, there has been known one disclosed, for instance, in Japanese Patent Publication No. 7(1995)-119893. The objective optical system is provided with an adjustable diaphragm and when a part relatively close to the optical system is to be observed, the diaphragm is closed to increase the depth of focus.

However since fluorescence emitted from a part inside the body is very weak as described above, the method of increasing the depth of focus by closing the diaphragm is difficult to apply to the fluorescence endoscope.

In order to overcome the aforesaid problem, we have proposed to carry out, in an endoscope comprising an objective optical system which is inserted into the interior of an organic body and an image taking means which takes an image formed by the objective optical system and outputs an image signal representing the image, an image restoration processing on a part of the image signal corresponding to a predetermined range in the image taking range of the image taking means by use of a degradation function such as a point spread function of the objective optical system. See Japanese Unexamined Patent Publication No. 10(1998)-165365.

In the endoscope, blur of an image due to insufficient depth of focus of the objective optical system can be removed by an image restoration processing using a degradation function such as a point spread function or the like of the objective optical system. When blur of the image due to insufficient depth of focus of the objective optical system can be avoided in this manner, a high numerical aperture objective optical system can be employed and accordingly the amount of light entering the image taking means can be increased.

However in the endoscope, since a preset design value or a measured value is employed as the degradation function, it is difficult to accurately restore the image taking into account change of the degradation function which is caused, for instance, when the position of the endoscope relative to the part to be observed changes.

The problem may be overcome, when the endoscope is arranged to measure the distance between each point on the part to be observed and the endoscope and to carry out the image restoration processing on a part of the image signal corresponding to the part of the body whose distance from the endoscope is within a predetermined range. However this approach is disadvantageous in that it requires a distance measuring means, which complicates the structure of the endoscope.

SUMMARY OF THE INVENTION

In view of the foregoing observations and description, the primary object of the present invention is to provide an endoscope in which the image restoration processing can be accurately carried out even if the position of the endoscope relative to the part to be observed changes without use of a distance measuring means.

In accordance with the present invention, there is provided a fluorescence endoscope comprising

an illuminating light projecting system which projects illuminating light onto a part inside a body,

a first objective optical system which is inserted into the interior of the body and causes the illuminating light reflected at the part of the body to form a normal image of the part,

a normal image taking means which takes the image formed by the objective optical system and outputs a normal image signal representing the normal image,

an excitation light projecting system which projects onto a part inside the body excitation light having a wavelength in the excitation wavelength range of a photosensitive material,

a second objective optical system which causes fluorescence emitted from the part to form a fluorescence image, and

a fluorescence image taking means which takes the fluorescence image formed by the second objective optical system and outputs a fluorescence image signal representing the fluorescence image,

wherein the improvement comprises

a measuring light projecting system which projects measuring light having a wavelength in the wavelength range of the fluorescence onto a part inside the body,

an operation means which obtains an image degradation function of the second objective optical system through operation using image signals respectively output from the normal image taking means and the fluorescence image taking means on the basis of images formed through the first and second objective optical systems by the measuring light reflected at the same part of the body, and

an image processing means which carries out an image restoration processing on a fluorescence image signal output from the fluorescence image taking means on the basis of the image degradation function.

As the measuring light projecting system, those which emit light substantially equal in spectrum to the fluorescence emitted from the part of the body upon exposure to the excitation light or light having a wavelength substantially equal to a main peak wavelength of the fluorescence can be suitably employed.

As the degradation function, a point spread function of the second objective optical system can be suitably used.

The first and second objective optical systems may be formed either separately from each other or as a single objective optical system common to the normal image taking means and the fluorescence image taking means.

However when the first and second objective optical systems are formed separately from each other, parallax between the objective optical systems can cause distortion of the image. Accordingly, in such a case, it is preferred that the endoscope be further provided with a means for carrying out processing for correcting distortion of the image due to parallax between the first and second objective optical systems on the image signals respectively output from the normal image taking means and the fluorescence image taking means.

In the fluorescence endoscope in accordance with the present invention, images formed through the first and second objective optical systems by the measuring light reflected at the same part of the body are taken by the normal image taking means and the fluorescence image taking means, and an image degradation function of the second objective optical system is obtained by performing operation on the basis of the image signals output from the normal image taking means and the fluorescence image taking means. Since the degradation function thus obtained is based on data on images which are actually taken, the degradation function precisely reflects the actual position of the endoscope relative to the part to be observed. Accordingly provided that the position of the endoscope relative to the part to be observed when taking a fluorescence image is held unchanged from that when the degradation function is obtained, an extremely accurate image restoration processing can be carried out using a degradation function which precisely reflects the actual position of the endoscope relative to the part to be observed, whereby blur of an image due to insufficient depth of focus of the objective optical system can be well removed.

Further in the fluorescence endoscope of the present invention, since there is required no distance measuring means, the structure of the endoscope can be relatively simple.

It has been known that blur of an image can be effectively removed by an image restoration processing using a point spread function or the like of the objective optical system as a degradation function. Degradation functions of images can be roughly divided into those which represent degradation by a point spread function which can be analytically determined and those which represent degradation by a point spread function which cannot be analytically determined. In the present invention, the degradation by the point spread function can be known by actually measuring the input and output of the objective optical system and accordingly a degradation function in the former group can be suitably used. In this case, the image can be restored by de-convolution.

When the image is restored by the deconvolution, a filtering processing is generally applied. At this time, when noise can be ignored, the image can be restored by applying a so-called reverse filter to Fourier transform of the degraded image.

On the other hand, when noise cannot be ignored, the image can be restored by use of a least square filter (wiener filter) which minimizes the mean square error between the original image and the restored image, a limited reverse convolution filter, a recursive filter, a homomorphism filter, or the like.

Such an image restoration processing is described in detail, for instance, in papers of "Academy of Electronic Communication", November, 1984, Vol. J67-D, No.10, and "O plus E" magazine", an extra number, November, 1986. All the image restoration processings described there can be used in the present invention.

BRIEF DESCRIPTION OF THE DRAWING

FIG. **1** is a schematic side view of a fluorescence endoscope in accordance with an embodiment of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

In FIG. **1**, a fluorescence endoscope in accordance with an embodiment of the present invention comprises an excitation light source **10** such as a SHG laser which generates excitation light L1, for instance, in a blue region, a condenser lens **11** which condenses the excitation light L1 and a light guide **12** which is formed of optical fibers and is disposed so that the condensed excitation light L1 enters the light guide **12**. The endoscope is further provided with an illuminating light source **13** which emits white illuminating light L2 in a direction perpendicular to the optical path of the excitation light L1, a filter wheel **14** which is disposed on the optical path of the excitation light L1 at 45° thereto, and a drive means **15** which rotates the filter wheel **14**.

The filter wheel **14** is provided with a mirror **14***a* and a dichroic mirror **14***b* fixed thereto. The mirror **14***a* reflects the illuminating light as it is toward the condenser lens **11**. The dichroic mirror **14***b* reflects only a component of the illuminating light L2, which is white light, having a wavelength substantially equal to a peak wavelength of fluorescence L3 to be described later toward the condenser lens **11** as measuring light L2'. Further the filter wheel **14** is provided with an opening through which the excitation light L1 passes the filter wheel **14** to impinge upon the condenser lens **11**.

The filter wheel **14** is driven by the drive means **15** and is selectively positioned in one of a first position where the opening is on the optical path of the excitation light L1 so that the excitation light L1 passes the filter wheel **14** and impinges upon the condenser lens **11**, a second position where the mirror **14***a* reflects the illuminating light L2 toward the condenser lens **11** and a third position where the

dichroic mirror 14*b* reflects the measuring light L2' toward the condenser lens 11.

The light guide 12 is contained in a forceps passage 17B in a flexible probe 17 which is inserted into a body 16. A beam splitter 19 is disposed in the probe 17 and an objective lens 20 is disposed forward of the beam splitter 19.

The beam splitter 19 reflects downward a part of light impinging thereon and transmits the other part of the light as will be described later. A excitation light cut filter 21 and a fluorescence image taking means 22 are disposed in this order below the beam splitter 19. The fluorescence image taking means 22 may comprise, for instance, a CCD, and the fluorescence image taking means 22 is connected to an image processing system 23 and an image display system 24 which may comprise, for instance, a CRT. The light passing through the beam splitter 19 impinges upon a normal image taking means 25, which may comprise, for instance, a CCD. The normal image taking means 25 is also connected to the image processing system 23 and the image display system 24.

Operation of the fluorescence endoscope will be described hereinbelow. A photosensitive material which has affinity to tumor and emits fluorescence when excited by light has been absorbed by a diagnostic part 30 of the body 16. The photosensitive material may be, for instance, porphyrin. When the excitation light L1 is projected onto the diagnostic part 30, the photosensitive material emits fluorescence L3 and when the illuminating light L2 or the measuring light L2' is projected onto the diagnostic part 30, the illuminating light L2 or the measuring light L2' is reflected by the diagnostic part 30.

When a normal image is to be observed, the illuminating light source 13 is energized, and the filter wheel 14 is rotated to the second position where the mirror 14*a* reflects the illuminating light L2 toward the condenser lens 11. The illuminating light L2 emitted from the illuminating light source 13 is condensed by the condenser lens 11 and enters the light guide 12. The illuminating light L2 propagates through the light guide 12 and emanates from the front end of the light guide 12 to illuminate the diagnostic part 30.

A part of the illuminating light L2 reflected by the diagnostic part 30 passes through the beam splitter 19 to impinge upon the normal image taking means 25. At this time, a normal image of the diagnostic part 30 by the reflected illuminating light L2 is formed on the normal image taking means 25 by the objective lens 20, and the normal image is taken by the normal image taking means 25. An image signal S2, representing the normal image, output from the normal image taking means 25 is input into the image display means 24 and the normal image is displayed by the image display means 24.

An adjustable diaphragm 26 is disposed between the objective lens 20 and the beam splitter 19 and is closed to a predetermined diameter to increase the depth of focus of the objective lens 20 when the normal image is taken.

When a fluorescence image is to be taken, the excitation light source 10 is energized and the filter wheel 14 is rotated to the first position where the opening is on the optical path of the excitation light L1 so that the excitation light L1 passes the filter wheel 14 and impinges upon the condenser lens 11. The excitation light L1 emitted from the excitation light source 10 is condensed by the condenser lens 11 and enters the light guide 12. The excitation light L1 propagates through the light guide 12 and emanates from the front end of the light guide 12 to impinge upon the diagnostic part 30.

When the excitation light L1 is projected onto the diagnostic part 30, the photosensitive material which has been absorbed by the diagnostic part 30 emits fluorescence L3. A part of the fluorescence L3 is reflected by the beam splitter 19 to impinge upon the fluorescence image taking means 22. At this time a fluorescence image of the diagnostic part 30 by the fluorescence L3 is formed on the fluorescence image taking means 22 by the objective lens 20 and the fluorescence image is taken by the fluorescence image taking means 22. The excitation light L1 which is reflected by the diagnostic part 30 and travels toward the fluorescence image taking means 22 is cut by the excitation light cut filter 21.

An image signal S1, representing the fluorescence image, output from the fluorescence image taking means 22 is input into the image display means 24 and the fluorescence image is displayed by the image display means 24. Since the photosensitive material has affinity to tumor, only an image of the tumor part is basically displayed.

When the fluorescence image is taken, the adjustable diaphragm 26 is full opened so that the weak fluorescence L3 enters the fluorescence image taking means 22 as much as possible. When the adjustable diaphragm 26 is full opened, the depth of focus of the objective lens 20 is reduced and blur is apt to be generated in a part of the fluorescence image taken. Removal of such blur will be described hereinbelow.

The fluorescence image signal S1 output from the fluorescence image taking means 22 is also input into the image processing system 23 and is subjected to an image restoration processing using a point spread function of the objective lens 20. Specifically a deconvolution processing using the aforesaid various filters is carried out as the image restoration processing.

Out of such processings, a typical processing using a so-called reverse filter will be described below.

When a coordinate system for the whole image is expressed by (x, y) and a coordinate system for a part of the image is expressed by (x', y') while the original image and the degraded image are expressed respectively by f(x, y) and g(x, y), the following relation holds.

$$g(x,y)=\int\int h(x,y,x',y')f(x',y')dx'dy'+n(x,y)$$

wherein, h(x, y, x', y') is a degradation function, and n(x,y) is noise. When there is no noise, a degraded image of a point light source represented by f(x', y')=δ(x'−α), y'−β) is h(x, y, α, β). Accordingly h(x, y, α, β) is a point spread function which is independent of the position (α, β) of a point on the original image.

If a degraded image of a point does not exists in the position of the point except translation, the above relation is expressed by convolution as follows.

$$g(x,y)=\int\int h(x-x',y-y')f(x',y')dx'dy'+n(x,y)$$

When it is assumed that noise is negligible, the above formula may be as follows.

$$g(x,y)=\int\int h(x-x',y-y')f(x',y')dx'dy'$$

When taking Fourier transforms of the both sides of this formula and convolution theorem is applied thereto, the following formula holds.

$$G(u,v)=H(u,v)F(u,v)$$

wherein F(u, v), G(u, v) and H(u, v) are Fourier transforms of f(x, y), g(x, y) and h(x, y), respectively, and H(u, v) is a transfer function of a system for converting an original image f(x, y) to a degraded image g(x, y).

By taking a reverse Fourier transform of G(u, v)/H(u, v), the original image can be restored. Accordingly 1/H(u, v) is referred to as "a reverse filter".

The image signal S1' which has been subjected to the image restoration processing is input into the image display means **24** and a fluorescence image is displayed by the image display means **24** on the basis of the image signal S1'. In this particular embodiment, the fluorescence image is displayed on the image display means **24** together with a fluorescence image reproduced on the basis of the original image signal S1. This arrangement allows the restored image to be compared with the original image, whereby the effect of the image restoration processing can be confirmed.

Further in the embodiment described above, the command for carrying out the image restoration processing on the image signal S1 is manually input by use of an input means **27** such as a keyboard. Accordingly, whether the image restoration processing is to be carried out can be selected.

However since blur is apt to be generated when the adjustable diaphragm **26** is in full open, it is possible to input the control signal of the adjustable diaphragm **26** also into the image processing system **23** and to automatically carry out the image restoration processing when the diaphragm **26** is full opened. This simplifies the operation of the operator.

Determination of the point spread function of the objective lens **20**, i.e., the degradation function h(x, y, x', y'), will be described hereinbelow. When determining the point spread function, the illuminating light source **13** is energized and the filter wheel **14** is rotated to the third position where the dichroic mirror **14***b* reflects the measuring light L2' toward the condenser lens **11**. The component of the illuminating light L2 having a wavelength substantially equal to a peak wavelength of fluorescence L3 is reflected by the dichroic mirror **14***b* toward the condenser lens **11** as measuring light L2'. The measuring light L2' is condensed by the condenser lens **11** and enters the light guide **12**. The measuring light L2' propagates through the light guide **12** and emanates from the front end of the light guide **12** to impinge upon the diagnostic part **30**.

A part of the measuring light L2' reflected by the diagnostic part **30** passes through the beam splitter **19** and impinges upon the normal image taking means **25**. At this time, an image (measurement image) of the diagnostic part **30** by the measuring light L2' is formed on the normal image taking means **25** by the objective lens **20**, and the measurement image is taken by the normal image taking means **25**. An image signal S2, representing the measurement image, output from the normal image taking means **25** is input into the image processing system **23**.

When the measurement image is taken, the adjustable diaphragm **26** is kept closed.

Thereafter, the adjustable diaphragm **26** is full opened and an measurement image is taken by the fluorescence image taking means **22**. Also, in this case, the measuring light L2' is projected onto the diagnostic part **30** and a part of the measuring light L2' reflected by the diagnostic part **30** is reflected by the beam splitter **19** to impinge upon the fluorescence image taking means **22**. At this time, a measurement image of the diagnostic part **30** by the measuring light L2' is formed on the fluorescence image taking means **22** by the objective lens **20**, and the measurement image is taken by the fluorescence image taking means **22**. An image signal S1, representing the measurement image, output from the fluorescence image taking means **22** is input into the image processing system **23**.

In this example, since the images taken by the normal image taking means **25** and the fluorescence image taking means **22** are formed by the same objective lens **20**, the image signals S2 and S1 represent images of the same part.

The image processing system **23** calculates a deterioration function h(x, y, x', y') for each position (x, y) on the basis of the image signals S1 and S2 thus input into the image processing system **23**. The image processing system **23** stores the deterioration functions h(x, y, x', y') thus obtained in a built-in memory and reads out the same when the aforesaid image restoration processing is to be carried out.

The processing of obtaining the deterioration functions h(x, y, x', y') is carried out just before a fluorescence image is taken and displayed. When the fluorescence image is taken and displayed, the position of the objective lens **20** relative to the diagnostic part is held unchanged from that when the degradation functions are obtained. In this manner, an extremely accurate image restoration processing can be carried out using the degradation functions h(x, y, x', y') which precisely reflect the actual position of the objective lens **20** relative to the diagnostic part **30**.

In general clinical treatment and the like, a step of displaying a normal image of a tumor suspect portion and observing the same, a step of taking the measurement images with the adjustable diaphragm full opened and obtaining the degradation functions, and a step of taking a fluorescence image and restoring the fluorescence image are performed in sequence.

When a fluorescence image is taken and displayed, unevenness on the diagnostic part **30** makes ununiform the amount of the excitation light L1 to which each portion of the diagnostic part is exposed, which results in fluctuation in the intensity of the fluorescence L3. The fluctuation in the intensity of the fluorescence L3 due to ununiformity of the excitation light L1 cannot be distinguished from that due to fluctuation in the concentration of the photosensitive material, that is, fluctuation in the intensity of the fluorescence L3 depending on the condition of the tumor.

In such a case, by taking a measurement image of the diagnostic part **30** by the measuring light L2, just before taking a fluorescence image and normalizing a fluorescence image signal S1' on the basis of the image signal representing the measurement image, the components due to ununiformity of the excitation light L1 can be removed.

In the embodiment described above, a single objective lens **20** is used both for forming the normal image and the fluorescence image, and the normal image and the fluorescence image are taken by separate image taking means **25** and **22**.

However the normal image and the fluorescence image may be formed by separate objective optical systems. In this case, parallax between the objective optical systems can cause distortion of the image. Accordingly, in such a case, it is preferred that the image signals S1 and S2 output from the fluorescence image taking means **22** and the normal image taking means **25** when the measurement images are taken be subjected to processing for correcting distortion of the images due to parallax between the objective optical systems. For example, the correction can be performed by coordinate transformation using correction data depending on distortion of the images due to parallax which are stored in a look-up table.

Further in the fluorescence endoscope of the present invention, both an objective optical system and an image taking means may be used for taking a normal image and taking a fluorescence image. However, even in this case, it is impossible for the single image taking means to take measurement images through the normal image taking system and the fluorescence image taking system at one time.

Accordingly, it is necessary to take a measurement image through the normal image taking system with the adjustable diaphragm closed and then take a measurement image through the fluorescence image taking system with the adjustable diaphragm full opened.

Though, in the embodiment described above, a photosensitive material is given to the diagnostic part in advance, the present invention may be applied to a case where a fluorescence image formed by auto-fluorescence emitted from the intrinsic photosensitive material is taken.

What is claimed is:

1. A fluorescence endoscope comprising

an illuminating light projecting system which projects illuminating light onto a part inside a body,

a first objective optical system which is inserted into the interior of the body and causes the illuminating light reflected at the part of the body to form a normal image of the part,

a normal image taking means which takes the image formed by the objective optical system and outputs a normal image signal representing the normal image,

an excitation light projecting system which projects onto a part inside the body excitation light having a wavelength in the excitation wavelength range of a photosensitive material,

a second objective optical system which causes fluorescence emitted from the part to form a fluorescence image, and

a fluorescence image taking means which takes the fluorescence image formed by the second objective optical system and outputs a fluorescence image signal representing the fluorescence image,

wherein the improvement comprises

a measuring light projecting system which projects measuring light having a wavelength in the wavelength range of the fluorescence onto a part inside the body,

an operation means which obtains an image degradation function of the second objective optical system through operation using image signals respectively output from the normal image taking means and the fluorescence image taking means on the basis of images formed through the first and second objective optical systems by the measuring light reflected at the same part of the body, and

an image processing means which carries out an image restoration processing on a fluorescence image signal output from the fluorescence image taking means on the basis of the image degradation function.

2. A fluorescence endoscope as defined in claim 1 in which the measuring light projecting system emits light substantially equal in spectrum to the fluorescence emitted from the part of the body upon exposure to the excitation light.

3. A fluorescence endoscope as defined in claim 1 in which the measuring light projecting system emits light having a wavelength substantially equal to a main peak wavelength of the fluorescence emitted from the part of the body upon exposure to the excitation light.

4. A fluorescence endoscope as defined in claim 1 in which the degradation function is a point spread function of the second objective optical system.

5. A fluorescence endoscope as defined in claim 1 in which the first and second objective optical systems are formed separately from each other and there is provided a means for carrying out processing for correcting distortion of the image due to parallax between the first and second objective optical systems on the image signals respectively output from the normal image taking means and the fluorescence image taking means.

* * * * *

NEW METHODS & MATERIALS

Fluorescein electronic endoscopy: a novel method for detection of early stage gastric cancer not evident to routine endoscopy

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Background: **Fluorescence endoscopy with fluorescein sodium in the stomach was evaluated by using a newly developed fluorescence electronic endoscopic system.**
Methods: **Sixteen patients with early stage gastric cancer diagnosed by white light endoscopy and chromoendoscopy underwent fluorescein electronic endoscopy before surgery. The resection specimens underwent thorough histopathologic evaluation.**
Results: **About 10 seconds after intravenous injection of fluorescein, fluorescence appeared and immediately spread throughout the gastric surface. A few minutes later, differentiated early stage gastric cancers with more abundant stroma than surrounding normal mucosa exhibited significantly stronger fluorescence, and those with less stroma exhibited weaker fluorescence than the surrounding normal mucosa. Undifferentiated early stage gastric cancers, in which the stroma became wider because foveolae were collapsed from malignant invasion, expressed stronger fluorescence intensity. In all cases, the borders of early stage gastric cancers were clearly demonstrated. Among the 16 patients, 6 accompanying flat lesions and 1 tiny lesion not evident by routine endoscopy were detected. The extent of the cancers, as determined by fluorescence endoscopy, were similar to those determined histopathologically.**
Conclusions: **Fluorescein electronic endoscopy is useful in determining the extent within the mucosa of gastric cancers when this is obscure by standard endoscopic observation, and for detecting extremely early stage cancer that is not evident by conventional endoscopic observation.**

Therapeutic endoscopic methods, such as endoscopic mucosal resection, laser irradiation, microwave coagulation, and electrosurgical resection of polypoid early stage gastric cancers, have become increasingly important as the population in many societies ages.[1-3] However, there are several problems with the use of these therapeutic modalities in the treatment of GI cancer: (1) The exact extent of cancer within the mucosa must be known to avoid the problem of residual cancer after treatment. (2) To obtain a cure, the lesion must be detected and treated while it is still small. (3) The detection rate of minute flat lesions, most of which are not evident to routine endoscopy, must be improved.

Double-contrast radiography and conventional endoscopy together with chromoscopy (dye-spraying techniques) are effective in detecting cancerous lesions that are elevated above the plane of the adjacent normal mucosa (types I and IIa, Japanese classification of gastric carcinoma) or depressed relative to the adjacent normal mucosa (types IIc and III). However, there is still no effective technique for detection of, and determination of the extent of, flat lesions level with nearby normal mucosa (type IIb).

In carcinomatous tissue, cellular and structural atypia of the epithelium should be accompanied by changes in the stroma including internal vascular structures. Therefore, by contrasting the abnormal vascular structure, the area of the mucosa that contains a gastric malignancy, including the early stage flat type cancer, could be clearly revealed. The aim of the present study was to evaluate a new type of endoscopy based on this hypothesis, by means of which it may be possible to precisely detect the extent of early stage gastric cancers within the mucosa.

To test this hypothesis, black ink was injected in a pilot study into the main gastric arteries associated with resected stomachs. Fluorescent substances can also be used to contrast vascular structures in vivo. Fluorescein sodium is a widely used in ophthalmology to show the fine vascular structure of the retina. By using a fiberscope, Katsu et al.[4-6] succeeded in 1975-1976 in observing and recording the fluorescence of fluorescein sodium on the stomach surface. However, fluorescein fiberoptic endoscopy has a clear disadvantage: the images are dark. A new fluorescence electronic endoscopic system was developed that produces bright, high resolution images. With this system, changes in fluorescence within the gastric and colonic mucosa can be observed in real time after intravenous injection of fluorescein sodium. The present study, using this new fluorescence electronic endoscopic system, documents the findings of fluorescein electronic endoscopy (FEE) in early stage gastric cancer.

PATIENTS AND METHODS

This protocol was approved by the ethics committee of our hospital.

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Contrasting gastric mucosal vascular structure with ink

Eleven resection specimens of human stomach obtained by total or subtotal gastrectomy performed for a diagnosis of early stage gastric cancer were used. Black ink was injected into either the left or right gastric artery after perfusion with heparinized saline solution. The resection specimens were fixed with 15% formalin overnight and then studied with a stereomicroscope (B071, Olympus Optical Co., Ltd., Tokyo, Japan). Subsequently, the specimens were studied with routine histopathologic methods. If necessary, to match the findings by stereomicroscopy and histopathologic examination after preparation of histologic sections, the paraffin blocks were resolved and tissue blocks were restudied with the stereomicroscope.

Methodology for fluorescence electronic endoscopic system

Stains of diluted fluorescein sodium on white paper were observed with a standard white light electronic endoscope system and with an electronic endoscope (GIF-XQ200, Olympus) with a barrier filter (Wratten Filter No. 15, Eastman Kodak Co., Rochester, N.Y.) attached to the objective lens. The observations were made without an excitor filter (Wratten Filter No. 47A, Kodak), a type of filter recommended for exciting fluorescein, and without an adjuster filter (light balancing, blue [LBB 200], Olympus), and without either of these filters being placed in front of the lamp in the light source. The light source and video processor used were, respectively, the CLV-U2OD (Olympus), and CV-200 (Olympus). Transmission characteristics of the adjuster, barrier, and excitor filters are shown in Figure 1.

FEE of early stage gastric cancer

The adjuster and barrier filters, electronic endoscope, light source, and video processor used in vivo were the same as those described above. Sixteen patients with histopathologically confirmed early stage gastric cancers found by white light endoscopy and chromoendoscopy underwent FEE before operation. Informed consent was obtained from all patients. An electronic endoscope with a barrier filter attached to the objective lens was passed to the stomach. After the lesion was identified, an adjuster filter was placed in front of the light source and a single intravenous injection of 10% fluorescein sodium of 5, 7, or 10 mL was made over a few seconds through a plastic catheter inserted into a forearm vein. Observations were then made with the blue channel of monitor being maximized and red channel minimized. Findings were recorded by videotape, color film, and a video color printer. Patients subsequently underwent operation and the resection specimens were examined by stereomicroscopy and standard light microscopy, and the findings compared with those by FEE.

RESULTS

Contrasting gastric mucosal vascular structure with ink

After perfusion of the mucosal vascular structures with ink, the borderlines between normal gastric mucosa and early stage gastric cancers, including differentiated and undifferentiated flat type tumors and even between different types of normal gastric mucosa, were clearly and precisely revealed. This was because the surface microstructure, that is, foveolae and interfoveolar processes, and vascular structures within the interfoveolar processes, of these tissues differed significantly, and thus there were different degrees of darkness in the mucosal surface. Mucosa with broad and large interfoveolar processes containing small numbers of blood vessels appeared whitish. In contrast, those with narrow and small interfoveolar processes containing large numbers of blood vessels appeared blackish. Even where undifferentiated cancer cells invaded the mucosal stroma only sparsely without causing any change in the surface foveolar pattern, the extent of the tumor in the mucosa was easily observed after the ink injection. This was because interfaveolar processes with stroma invaded by undifferentiated cancer cells became edematous, and thus looked more whitish compared with those without cancer cells.

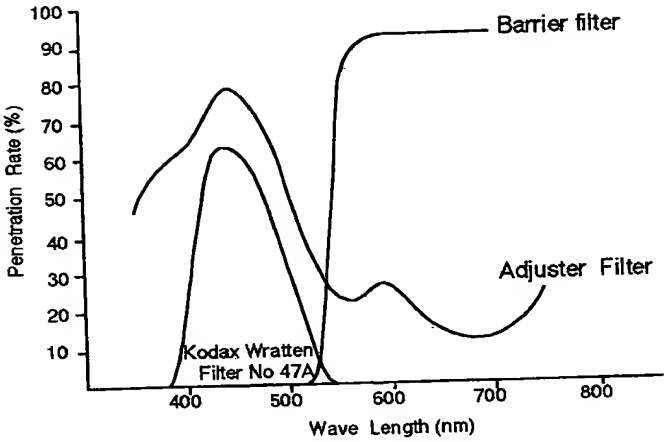

Figure 1. Graph showing transmission characteristics of adjuster, barrier, and excitor filters. The barrier filter blocks transmission of blue light completely and passes all of the green and red light. The adjuster filter passes mostly blue light and portions of green and red light. The excitor filter passes only blue light and completely blocks green and red light.

Case 1

Case 1 is a representative case of a gastric cancer, the extent of which was not correctly recognized by routine endoscopy. A 16 × 20 mm elevated lesion was found on the posterior wall of the antrum. Endoscopically, the entire lesion appeared homogenous (Fig. 2). However, in the ink-injected specimen, only the top area became whitish (Fig. 3). Under high magnification stereomicroscopy, a clear-cut borderline between the whitish and blackish parts of the

Figure 2. Endoscopic view of a homogenous-appearing elevated lesion, about 16 × 20 mm, in antrum.

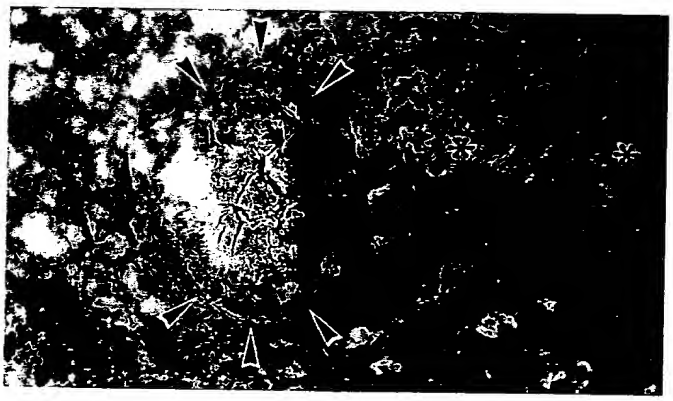

Figure 3. Photomicrograph of macroscopic appearance of same lesion as in Figure 1 after injection of ink into mucosal vascular structure (×2). *Arrowheads* indicate line where lesion is elevated from the gastric surface. The top of the lesion is whitish whereas the remaining portions appear blackish. On the smooth surface, there are also whitish areas *(asterisks)* confirmed to be intestinalized gastric mucosa. The blackish areas were confirmed to be pyloric gland mucosa.

lesion was observed (Fig. 4A). Histologic examination indicated that only the top whitish area was adenoma with moderate atypia whereas the blackish area was hyperplastic pyloric gland mucosa (Fig. 4B).

Methodology for fluorescence electronic endoscopic system

Under white light, the fluorescein sodium stains on white paper appeared with the endoscope as yellowish and without fluorescence (Fig. 5A). But, with the barrier filter alone (attached on objective lens), the fluorescein stains exhibited white fluorescence (Fig. 5B), which became strongest when the electronic signal of the blue channel of the monitor was

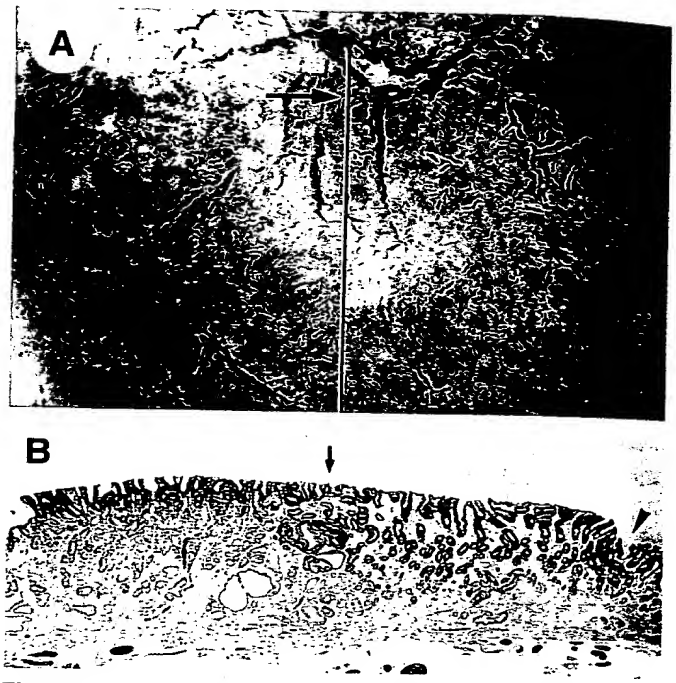

Figure 4. A, Stereomicroscopic photograph of lower portion of elevated lesion in Figure 3 (×10). Note clear-cut borderline between whitish and blackish area. **B,** Photomicrograph of histologic section cut on the line shown in **A** and observed from the left side *(arrow)* (H&E, orig. mag. ×12). The whitish area appears to be atypical epithelium of the type associated with adenoma, and blackish area appears to be hyperplastic pyloric gland mucosa. *Arrow* indicates borderline between these two. *Arrowhead* denotes place where entire lesion rises above gastric surface.

maximized (Fig. 5C) and weakest when the blue channel was minimized (Fig. 5D). Strengthening the red channel made the white paper look orange (Fig. 5E), whereas weakening this channel made the white paper look green (Fig. 5F). However, strengthening and weakening the red channel produce little influence in the strength of white fluorescence arising in the fluorescein stains (Fig. 5E and F). With the adjuster filter in front of the light source and barrier filter on the objective lens, the fluorescence of the fluorescein stain became brighter and its contour was sharper (Fig. 5G, compare with Fig. 5B). The white paper looked yellow, but dimmer compared with the image of the barrier filter alone (Fig. 5B and G). With excitor filter and barrier filter the fluorescence looked bluish with sharp outlines, but the white paper became dark and its contour could not be observed (Fig. 5H). The brightness of fluorescence obtained from the combination of excitor and barrier filter (Fig. 5H) was weaker than that obtained by the combination of adjuster and barrier filter or barrier filter alone (Fig. 5C).

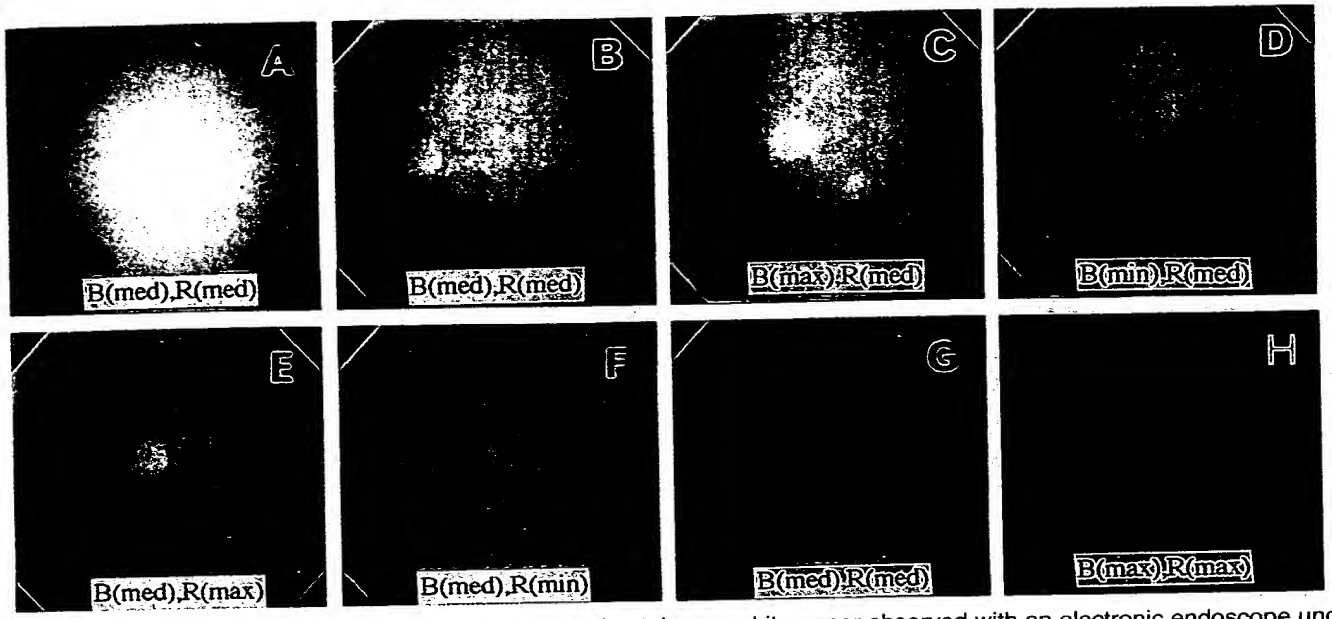

Figure 5. Photographs showing appearances of fluorescein stains on white paper observed with an electronic endoscope under varying conditions. **A,** White light; **B-F,** with barrier filter alone attached to objective lens; **G,** with an adjuster filter in front of light source and barrier filter; **H,** with an excitor filter in front of light source and a barrier filter. *B(max),B(med),B(min)*: strength of blue channel on monitor; maximum, medium, and minimum, respectively. *R(max),R(med),R(min)*: strength of red channel on monitor.

Figure 6. A, Fluorescence endoscopic view of minute flat gastric cancer exhibiting white fluorescence *(asterisk)* (5 mL fluorescein sodium) located near a depressed lesion; **B,** white light endoscopic photograph of same lesion as in **A**. No significant change is evident in the area indicated by asterisk in **A. C,** Stereomicroscopic photograph of same minute flat lesion *(asterisk)*. *Arrowhead* indicates edge of depressed lesion. Small arrows in **A, B,** and **C** denote the same line. A tissue section on the line in **C,** observed from right side *(large arrow)*, is demonstrated in Figure 7B.

FEE of early gastric cancers

Of the 16 patients, 13 had differentiated and 3 undifferentiated cancer. About 10 seconds after intravenous injection of fluorescein, fluorescence appeared as a white or white-blue light that immediately spread throughout the gastric surface. A few minutes later, differentiated early gastric cancers with more abundant stroma than surrounding normal mucosa exhibited significantly stronger fluorescence, and those with less stroma exhibited weaker fluorescence than the surrounding normal mucosa and looked reddish.

Undifferentiated early stage gastric cancers, in which the stroma became wider because foveolae were collapsed because of invasion by cancer, expressed a stronger fluorescence intensity. In all patients, the borderline of early stage gastric cancer could be clearly observed. Among the 16 patients, 6 additional flat lesions and 1 tiny lesion not seen by white light endoscopy and chromoendoscopy were detected. The extent of these lesions as demonstrat-

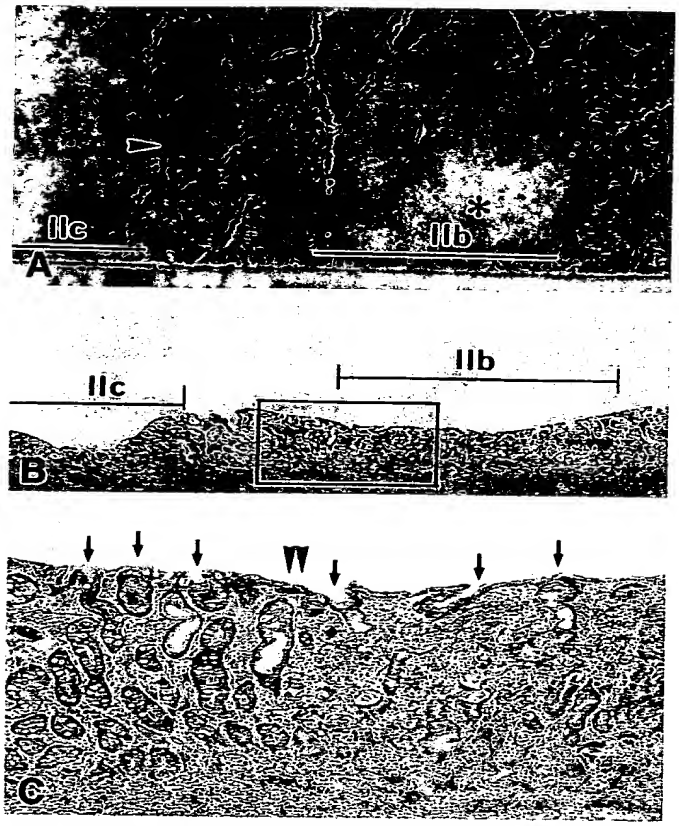

Figure 7. Same lesion as in Figure 6. **A,** Stereomicroscopic photograph of surface of tissue block after resolving the paraffin (methylene blue, orig. mag. ×15). *Arrowhead* indicates the edge of depressed lesion (IIc). IIb with bar denotes extent of the minute flat cancer *(asterisk).* **B,** Tissue section on the line drawn on Figure 6C observed from right side *(large arrow)* (H&E, orig. mag. ×15). IIc with bar indicates extent of depressed lesion, and IIb with bar denotes extent of the minute flat cancer (*asterisk* in 6A, 6C, 7A). **C,** Photomicrograph of rectangle in **B** showing borderline *(double arrowheads)* between the cancer *(right side)* and normal *(left side)* mucosa. Note that stroma between the foveolae *(arrows)* is more abundant in cancerous tissue than in normal mucosa (H&E, orig. mag. ×30).

ed by FEE was similar to the extent as determined by histologic examination. Two cases are presented as examples of FEE.

Case 2

A minute flat lesion showing white fluorescence under FEE was found adjacent to a depressed carcinoma on the anterior wall of the antrum (Fig. 6A). At the beginning, the fluorescence of this flat lesion was as strong as that of the surrounding normal mucosa, but after a few minutes the former became stronger whereas the latter faded away. By white light endoscopy and chromoendoscopy, this lesion did not exhibit any significant finding that suggested an abnormality (Fig. 6B). Its pit pattern observed by stereomicroscopy of the resected stomach showed mild disarray (Figs. 6C and 7A). Histopathologic examination indicated that the histologic type of this minute flat lesion as well as that of the main depressed lesion was differentiated adenocarcinoma with more abundant stroma than the surrounding normal mucosa (Fig. 7B and C).

Case 3

A slightly elevated lesion, 8 × 10 mm, at the gastric angle with a remarkably obscure outline under white light endoscopy (Fig. 8A) and chromoendoscopy (Fig. 8B) was observed twice by FEE with doses of 10 mL and 7 mL on different days. About 10 seconds after injection of fluorescein sodium, fluorescence first appeared from the lesion and soon thereafter in the surrounding mucosa. After the fluorescence appeared on the gastric surface, the horseshoe-shaped contour of the lesion became clear because of the contrasting effect of FEE. By using the 10 mL dose, the contrasting effect was strongly observed but the difference in fluorescence intensity between the lesion and surrounding normal mucosa was not significant (Fig. 8C). It took more than 10 minutes for the lesion to appear reddish, with weaker fluorescence intensity compared with the surrounding mucosa. By using the 7 mL dose of fluorescein sodium, a few minutes after injection the lesion became reddish and sharply demarcated from the surrounding mucosa (Fig. 8D). In addition, another small (3 × 4 mm) elevated lesion near the initially discovered lesion, which was not apparent to standard endoscopy, was first recognized under FEE as a reddish lesion against the white fluorescing surrounding mucosa (Fig. 8E). The contrasting effect was weaker compared with that obtained with the 10 mL dose. Stereomicroscopy of the resection specimen demonstrated exactly the same shape of these lesions as shown by FEE (Fig. 9). Histologically, these 2 lesions were well differentiated adenocarcinoma with high glandular density and little stroma (Fig. 10A and B; Fig. 11A and B). Interestingly, before injection of fluorescein sodium with the adjuster filter inserted in front of the light source and the barrier filter attached to the objective lens, the larger lesion could be observed as a dark area (Fig. 8F), which had the same shape as observed by FEE (Fig. 8D) and stereomicroscopy (Fig. 9), against a bright yellow area of normal mucosa. This was the same as the findings by auto-fluorescence (Fig. 8F).

DISCUSSION

It is extremely difficult to determine the extent of flat-type early stage gastric cancer with double-

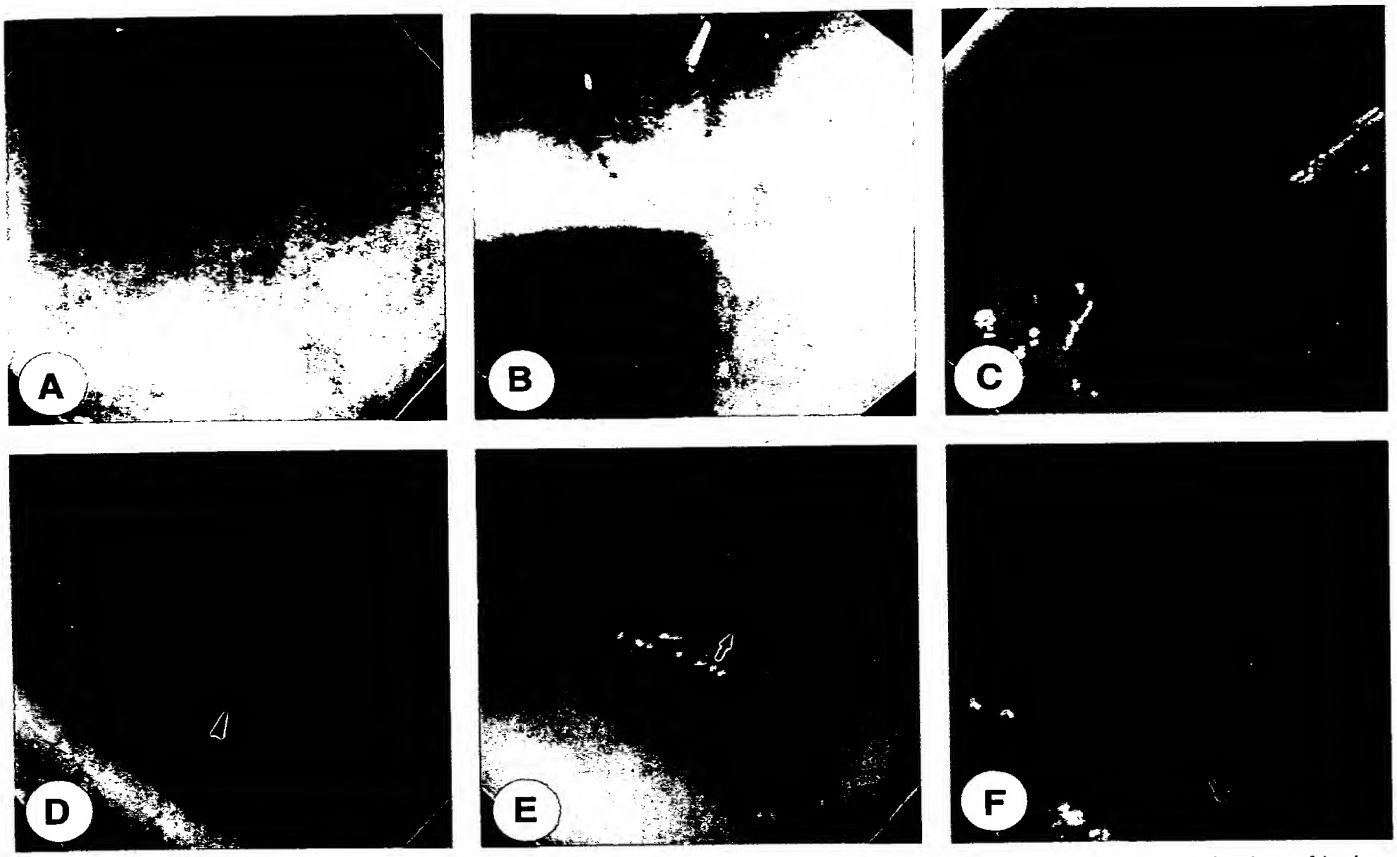

Figure 8. A, Conventional white light endoscopic view of elevated lesion at gastric angle. **B,** Chromoendoscopic view of lesion. Two markers have been placed proximally next to the lesion. **C,** Fluorescence endoscopic view (10 mL of fluorescein sodium). **D,** Fluorescence view (7 mL fluorescein sodium). **E,** Fluorescence view (7 mL fluorescein sodium). A smaller lesion *(arrowhead)* is now evident distal to the larger lesion *(arrow)* shown in **C** and **D**. **F,** Autofluorescence endoscopy view. The lesions are observed under light passing through an adjuster filter with an electronic endoscope with a barrier filter attached to the objective lens without injection of fluorescein sodium. The *horizontal line* across **E** and **F** is an artifact introduced by the video color printer. With the instruments used in the present study, which were adjusted for recording of findings under white light endoscopy, the FEE findings were more clearly observed in images produced by the video color printer rather than with color film.

contrast radiography and conventional endoscopic methods. However, increasing the contrast of the mucosal vascular architecture by injection of ink in the present study indicated that the extent of early gastric cancer, including differentiated and undifferentiated flat type, could be precisely defined. These findings strongly suggested that combining a vascular contrasting technique with endoscopy would be beneficial.

Fluorescein sodium is widely used to contrast retinal vascular structures of patients with retinopathy. The common side effects are mild, mainly yellow staining of skin and urine for a few days.[7] Serious adverse reactions are fortunately rare, with a frequency of 2 to 5 per 1000 angiograms. Fatal reactions have been reported, but occur with a frequency of less than 1 per 50,000 angiograms.[7]

Katsu et al.[4-6] described fluorescein fiberscope findings in the stomach in 83 patients including 5 with early stage and 14 with advanced gastric cancers. However, this technique had a decisive disadvantage in that the images were dim because of the weakness of the fluorescence emitted from tissue. To resolve this problem, fluorescence endoscopic systems available commercially use a combination of fiberscopes and intensified cameras, and thus special instruments are required. These systems are therefore expensive and difficult to operate.

Compared with other fluorescence endoscopic systems, the FEE system described here, which is based on commonly used electronic endoscopic instruments, is inexpensive and easy to use. The only additional items needed are a thin-filmed barrier filter (Wratten Filter No. 15, Kodak) and a glass adjuster filter (LBB 200, Olympus), which cost less than $30. For the present study, the barrier filter was attached to the glass covering the objective lens

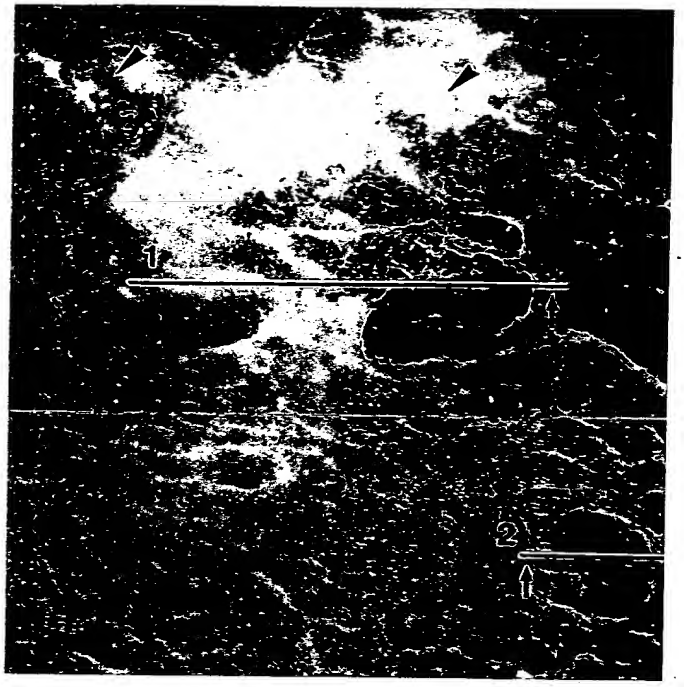

Figure 9. Stereomicroscopic photograph of lesions shown in Fig. 8. *Arrowheads* indicate sites of 2 markers seen in Fig. 8B (methylene blue, orig. mag. ×4).

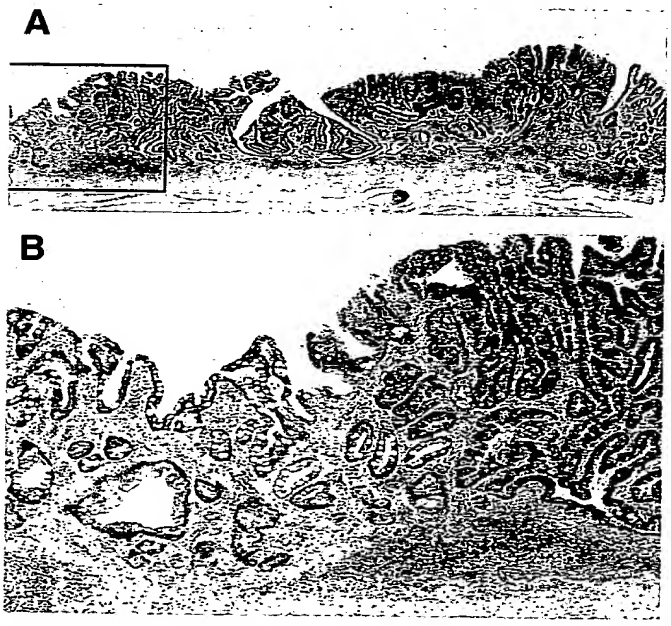

Figure 10. A, Photomicrograph of main lesion in Fig. 9 with section made on line 1 and observed from direction of the arrow (H&E, orig. mag. ×10). **B,** Photomicrograph at higher magnification (H&E, orig. mag. ×35) of rectangle in **A** showing borderline between the cancer and surrounding normal mucosa. Reflecting high glandular density, this tumor had less stroma compared with normal gastric mucosa.

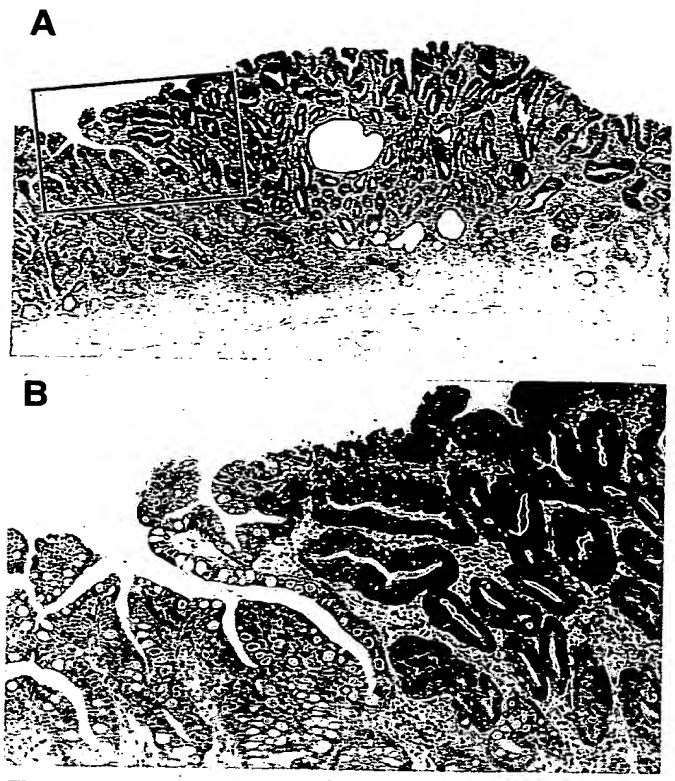

Figure 11. A, Photomicrograph of small lesion in Fig. 9 with section made on line 2 and observed from direction of arrow (H&E, orig. mag. ×25). **B,** Photomicrograph at higher magnification (H&E, orig. mag. ×85) of rectangle in **A,** showing borderline between the cancer and surrounding normal mucosa. Reflecting high glandular density, this tumor had less stroma compared with normal gastric mucosa.

of the endoscope with an instantaneous glue, and the adjuster filter was placed in the existing filter holder within the light source. The latter is moved with a single touch in or out of light path of the lamp. In the light source (CLV-U20D, Olympus), an RGB band pass filter is positioned in front of the lamp and is rotated to create light in the 3 primary colors, that is, red, green, and blue. Thus, tissue is illuminated sequentially by light with wavelengths that correspond to the 3 primary colors. A black and white charge-coupled device (CCD) behind the objective lens of the endoscope (GIF-XQ200, Olympus) picks up the photons of light reflected from tissue and generates electronic signals for, sequentially, red (R), green (G), and blue (B) reflected light. The video processor (CV-200, Olympus) receives these 3 electronic signals and reconstructs them on a color television monitor (Fig. 12).

When excited by blue light (465-490 nm), sodium fluorescein in solution emits a yellow-green fluorescence light (520-530 nm).[7] When viewed with the naked eye through the barrier filter, a fluorescein

stain on white paper under excitation with blue light appears as a yellow stain on dark paper because the barrier filter passes the fluorescence but not the blue light (Fig. 1). However, in the present study when observed with only the barrier filter attached to the objective lens of an electronic endoscope without excitation and adjuster filters, the fluorescein stains appeared as white fluorescence and the white paper appeared yellow on the television monitor (Fig. 5B). This can be explained as follows: the primary blue light from the light source activated the fluorescein sodium to yield yellow fluorescence and concomitantly was reflected almost completely from the white paper. The yellow fluorescence totally passed through the barrier filter whereas the blue light was completely blocked. The black and white CCD behind the barrier filter received only the yellow fluorescence from the fluorescein stain, which became an electronic signal in the B channel. Therefore, when looking at the B channel on the monitor, the fluorescein stains appear bright blue and the white paper appears dark. During the green (G) and red (R) phases of illumination, the reflected primary green and red light from the fluorescein stains and white paper are not markedly different in strength and the barrier filter does not block the green and red light (Fig. 12), thus on the G and R channels, both the fluorescein stain and the white paper appear bright. These observations can be confirmed for each channel by disconnecting the other 2 channels from the monitor. Reconstruction of the R, G, and B channels on the monitor results in an image that shows the stains to be white (bright blue + green + red) and the paper yellow (only green + red) (Fig. 13). As explained above, the fluorescent image was picked up by B channel. This is why the white fluorescence of the fluorescein stain appeared strongest when the electronic signal of the B channel was maximized (Fig. 5C) and appeared weakest when this signal was minimized (Fig. 5D). The adjuster filter used in the present study passes mostly blue light and a little green and red light (Fig. 1). When placed in front of the light source, it concentrates the blue light during the B phase of illumination and cuts off some portions of green and red light during the G and R phases. That is why, with a combination of adjuster and barrier filters, fluorescein stains exhibited stronger fluorescence and their outlines were emphasized, whereas the background, that is, the white paper, still appeared yellow (Fig. 5G) but dimmer than without the adjuster filter. The excitor filter selectively passed blue light but not green or red light (Fig. 1). Thus, with this filter and the barrier filter, during the B phase the fluorescein stain

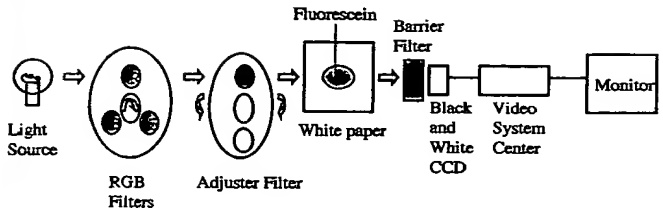

Figure 12. Schematic diagram of fluorescence electronic endoscopic system. White paper stained with fluorescein was used as the observed object.

appeared as bright blue and the white paper became dark; however, during the G and R phase both the fluorescein stains and white paper become dark because primary green and red light were completely blocked. Reconstruction of the 3 channels resulted only in bright blue stains on a dark background (Fig. 5H). On the final reconstructed image, the fluorescence obtained from the combination of excitor and barrier filters (Fig. 5H) was much dimmer than that obtained from the combination of adjuster and barrier filters, or the barrier filter alone (Fig. 5C).

The combination of the excitor and barrier filter is used in other fluorescence endoscopic systems. This explains why the images provided by these systems, which are usually composed of 2 colors (blue and red, or green and red), are still relatively dim, despite the use of an intensified CCD camera. By comparison, the system used in the present study can produce bright, high quality images even in an organ with a large internal volume, such as the stomach, where images tend to be dim. Real time changes in fluorescence displayed as white or white-blue light can be observed on a bright background. This is because the image provided by our FEE system is a coalescence of the fluorescent image, picked up and amplified electrically by the B channel, and the background image, which is picked up by the G and R channels with light adjusted by the adjuster filter; that is, the image is constructed with all 3 colors. Because the fluorescence image and background image are received by different channels, it is easy to recognize from where the fluorescence is emitted on the background.

Just as enhanced CT demonstrates space occupying lesions in the liver, brain, and other organs more clearly than plain CT, vascular contrasting endoscopy should reveal more precisely the extent of mucosal carcinoma. In the present study FEE detected 6 accompanying flat lesions and 1 tiny elevated lesion that were not evident by standard white light endoscopy and chromoendoscopy. These findings strongly suggest that FEE is useful in determining the extent of gastric cancer in the mucosa when this cannot be determined by stan-

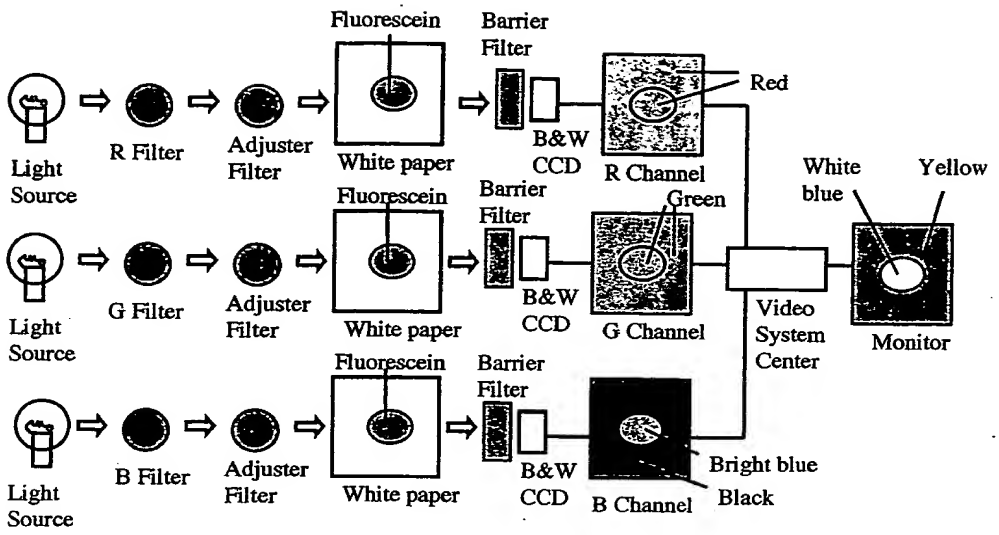

Figure 13. Schematic diagram illustrating design of fluorescence electronic endoscopic system.

dard techniques and in detecting extremely early stage cancers that are not evident by standard endoscopy.

The difference in fluorescence intensity between cancer and normal gastric tissue reflects the difference in the volume of fluorescein sodium within these tissues. Fluorescein sodium leaks from the blood vessels of all organs except the brain and eye. After a single injection into a vein, fluorescein sodium reaches the gastric surface and immediately leaks from capillaries into stroma and probably drains into lymphatics, which carry it away from the mucosa. Factors that may cause the difference in volume of fluorescein sodium in different types of gastric mucosa are differences in the stroma as well as vascular density and permeability. In the present study, which included 16 early stage gastric cancers, fluorescein accumulated to a greater extent in mucosa with abundant stroma and faded quickly from mucosa with less stroma. As described above, free fluorescein leaking from blood vessels into mucosal stroma plays an extremely important role in FEE and is responsible for the different intensities of fluorescence between normal and cancerous mucosa. Fluorescein, like other acidic substances, combines easily with serum proteins.[8] Protein-combined fluorescein does not leak from blood vessels and emits less fluorescence than free fluorescein.[8] Free fluorescein can be increased by increasing the amount of fluorescein sodium injected. However, in case 3, with a dose of 10 mL, a strong fluorescence intensity and contrasting effect was obtained, but the difference in fluorescence intensity between cancer and normal gastric mucosa was not so significant. In our experience, good results were usually obtained with a dose of 7 mL; overall fluorescence intensity was strong enough and the difference between a lesion and surrounding normal mucosa became clear a few minutes after injection. Further case studies are needed to determine the optimal dose of fluorescein sodium, which may depend on body weight and plasma protein level.

FEE appears to be a powerful method for determination of the precise extents of previously diagnosed lesions. Moreover, FEE detected small early stage gastric cancers, including differentiated (case 2 and 3) and undifferentiated (not shown) flat type cancer that were not apparent by standard white light endoscopy and chromoendoscopy. However, FEE findings per se are not diagnostic. For example, other than differentiated cancers with abundant stroma (case 2) or undifferentiated cancer, isolated intestinalized gastric mucosa and atrophic gastric mucosa, in which there are fewer glands and more abundant stroma, also exhibited a stronger fluorescence intensity than surrounding tissue. A benign area with a reddish depressed lesion-like appearance suggesting malignancy under white light endoscopy usually appeared similar under FEE to cancer, although with a more exaggerated appearance. Therefore, a biopsy is needed for definitive diagnosis. However, a finding of potential diagnostic value is the lesion, not evident by white light endoscopy, that becomes reddish by FEE with weaker fluorescence intensity compared with that of the surrounding mucosa. This finding implied that the lesion had small amounts of stroma, that is, had a high glandular density, the common characteristic of

adenoma and adenocarcinoma (case 3). In fact, 11 of the 16 early stage gastric cancers in the present study exhibited this finding under FEE. Note that the information provided by FEE is limited to the mucosa. In the present study, 2 cases of undifferentiated gastric cancer with the endoscopic appearance of early stage cancer were found microscopically to be invading the muscularis propria. FEE precisely demonstrated the extent of these cancers in the mucosa but provided no information as to depth of invasion.

Other fluorescence endoscopy systems have been developed for the detection of small cancers and the determination of their extent, such as photodiagnosis and autofluorescence endoscopy.[9-13] Photodiagnosis might prove to be the most sensitive technique for detection of minute cancers. The photosensitizers used in photodynamic diagnosis selectively localize within neoplastic tissue compared with surrounding normal tissue.[9-11] But patients must avoid strong light for a few weeks to prevent severe side effects caused by these photosensitizing substances.[14] Autofluorescence endoscopy does not require use of these agents and therefore has less potential for side effects and complications. However, in a study of autofluorescence endoscopy in stomach cancer with the light-induced fluorescence endoscopic imaging system, the detection rate for cancers limited to the mucosa was 57.5%, and mucosal thickening, as a result of cancer cell invasion, had a major impact on the detection rate.[15]

Compared with other methods and systems for fluorescence endoscopy, FEE is better suited to screening for early stage gastric cancers for the following reasons: (1) FEE images are bright and of high quality so that findings from the images are not smaller than those provided by white light endoscopy and chromoendoscopy. Moreover, FEE can demonstrate structural (i.e., stromal and vascular) differences between cancerous and normal mucosa as difference in fluorescent intensity, regardless of morphologic characteristics and color of tumors. FEE, therefore, reveals even flat-typed early stage gastric cancers that do not cause an appreciable change in color, and are usually not visible by white light endoscopy or chromoendoscopy. However, the FEE findings are not diagnostic of cancer and biopsies are still needed for definitive diagnosis. (2) By virtue of its long-standing use in other fields, ophthalmology in particular, fluorescein sodium is known to be an extremely safe agent. (3) The FEE system described here is inexpensive and easy to operate.

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DISCLOSURE

The device based on the methodology described in this work is under submission for Japanese, Chinese, European, and American patents.

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United States Patent [19]

Noguchi

[11] **Patent Number: 4,870,487**

[45] **Date of Patent: Sep. 26, 1989**

[54] **LIGHT SOURCE DEVICE FOR AN ENDOSCOPE WHICH MAINTAINS A CONSTANT MINIMUM-DC CURRENT**

[75] Inventor: **Toshiaki Noguchi, Tachikawa, Japan**

[73] Assignee: **Olympus Optical Co., Ltd., Tokyo, Japan**

[21] Appl. No.: **245,885**

[22] Filed: **Sep. 15, 1988**

Related U.S. Application Data

[62] Division of Ser. No. 119,604, Nov. 12, 1987, Pat. No. 4,800,424.

[30] **Foreign Application Priority Data**

Nov. 13, 1986 [JP] Japan 61-270461
Apr. 27, 1987 [JP] Japan 62-85528

[51] **Int. Cl.**[4] **H04N 7/18**
[52] **U.S. Cl.** **358/98**; 128/6
[58] **Field of Search** 358/98, 211, 42, 228, 358/901, 213.18; 128/4, 6

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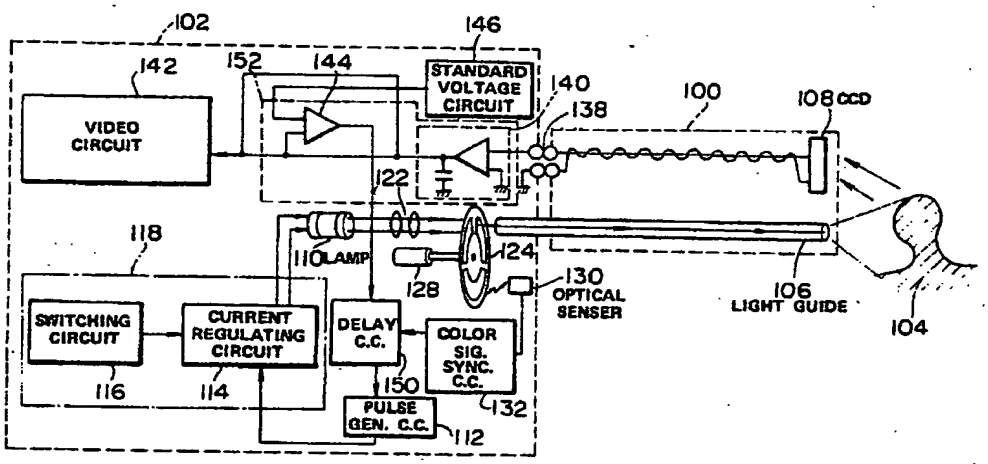
Primary Examiner—James J. Groody
Assistant Examiner—Victor R. Kostak
Attorney, Agent, or Firm—Armstrong, Nikaido, Marmelstein, Kubovcik & Murray

[57] **ABSTRACT**

A white light of a lamp, which can flash, is passed through a rotary filter fitted with a plurality of color filters to output field sequential type colored light. An output signal of a color filter detecting device, detecting the timing when the color filters are interposed in a light path, is used to produce trigger signals having different delay amounts. The light emitting timing of the lamp is delayed relatively from the time when the color filters are interposed in the light path to control the emitted light amounts of the colored light.

8 Claims, 8 Drawing Sheets



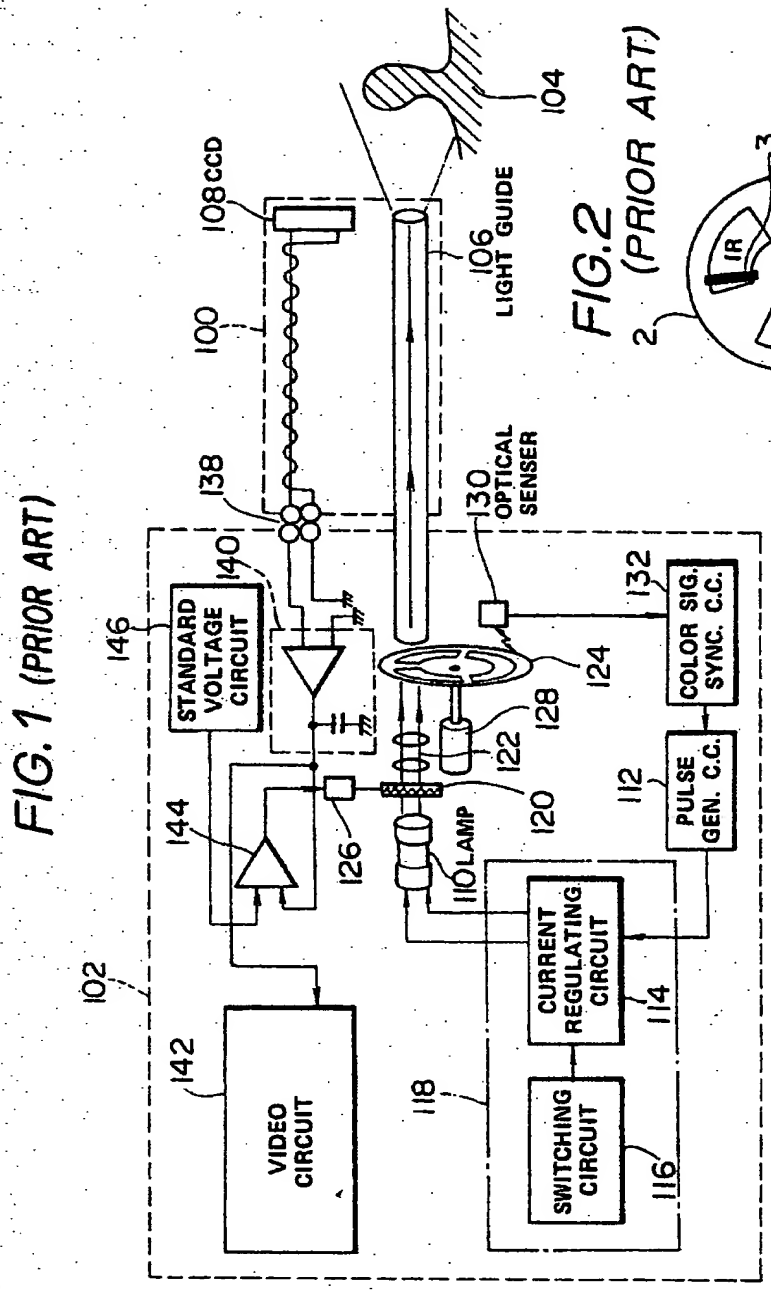


FIG. 1 (PRIOR ART)

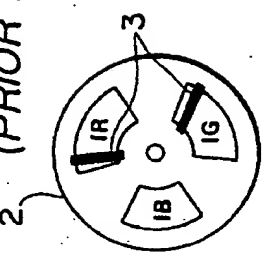
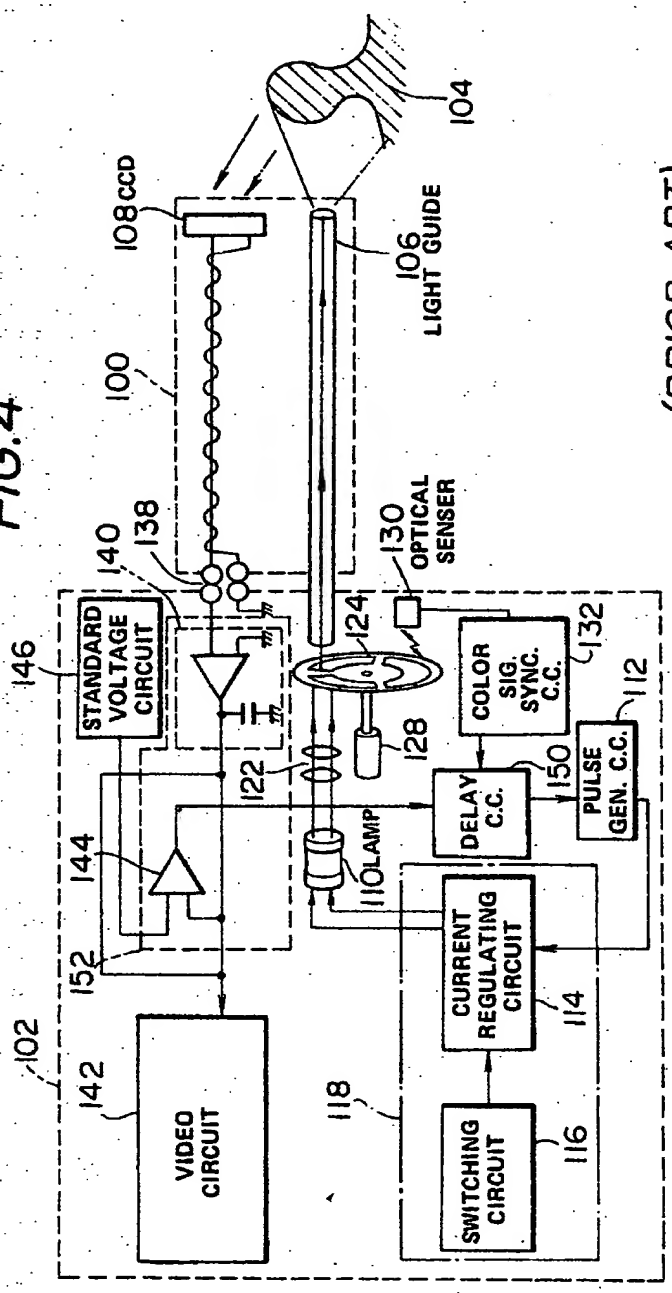


FIG. 2 (PRIOR ART)

FIG.4



(PRIOR ART)

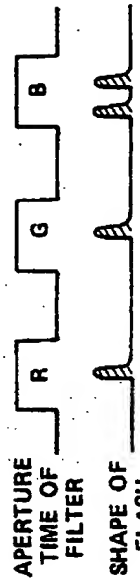
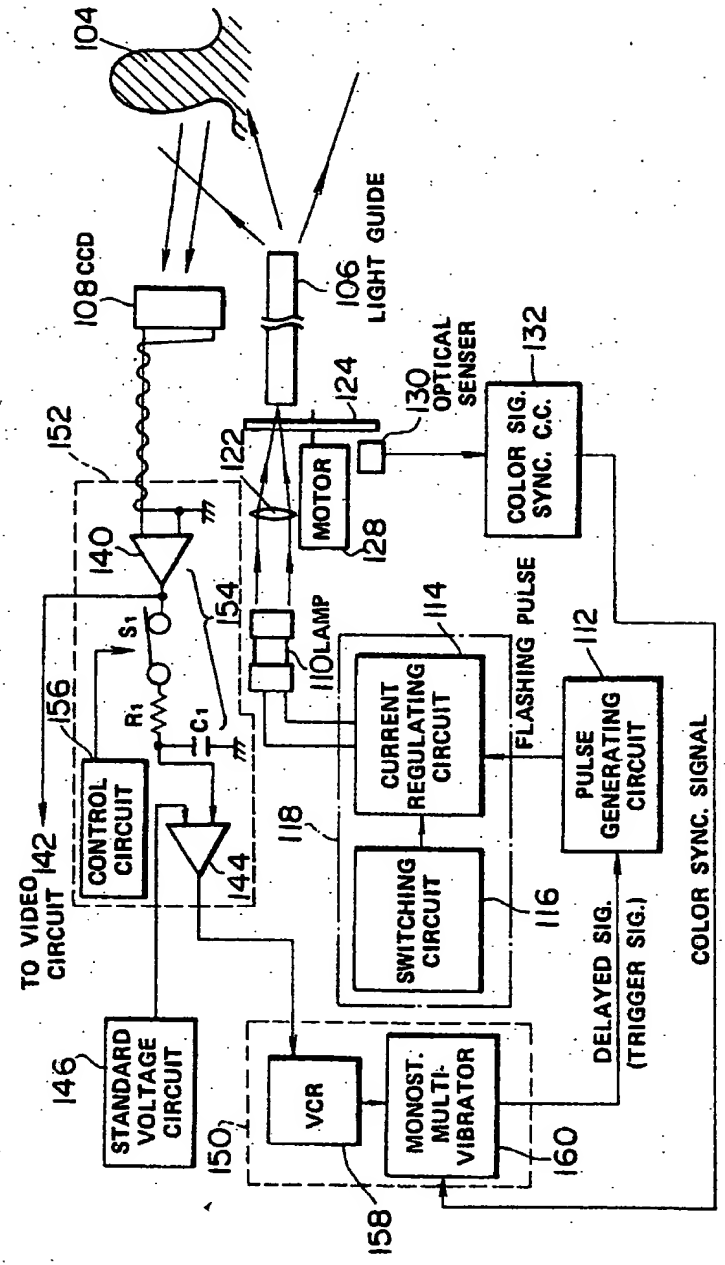


FIG.3a

FIG.3b

FIG.5


104
108 CCD
106 LIGHT GUIDE
124
130 OPTICAL SENSER
132
COLOR SIG. SYNC. C.C.
122
MOTOR
128
152
140
154
110 LAMP
114
CURRENT REGULATING CIRCUIT
FLASHING PULSE
112
PULSE GENERATING CIRCUIT
S1
R1
C1
156
CONTROL CIRCUIT
142
TO VIDEO CIRCUIT
144
118
SWITCHING CIRCUIT
116
DELAYED SIG. (TRIGGER SIG.)
COLOR SYNC. SIGNAL
146
STANDARD VOLTAGE CIRCUIT
150
VCR
MONOST. MULTI-VIBRATOR
160
158

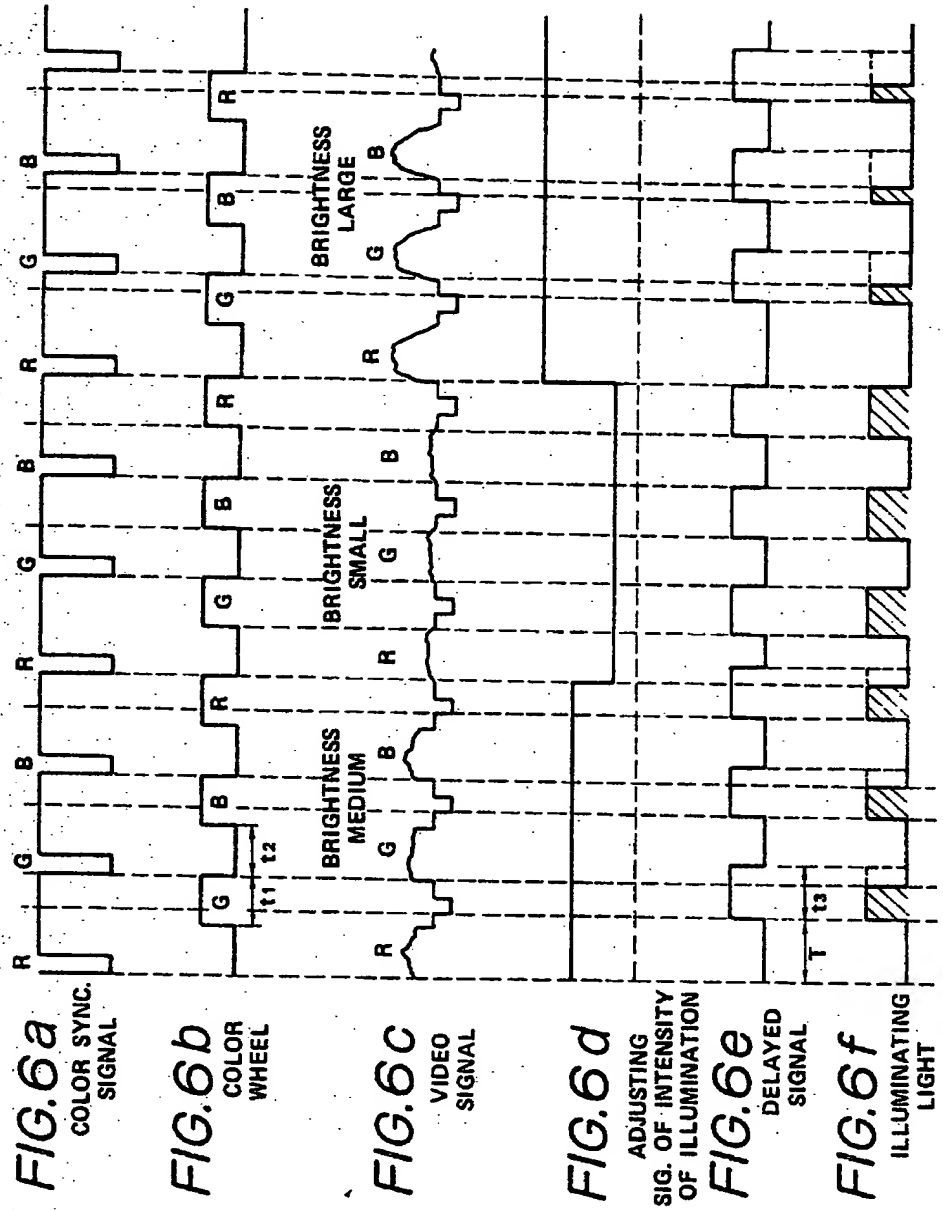

R
G
B
R
G
B
R
G
B
R
G
B
R
BRIGHTNESS LARGE
BRIGHTNESS SMALL
BRIGHTNESS MEDIUM
t1
t2
t3
T
FIG.6a COLOR SYNC. SIGNAL
FIG.6b COLOR WHEEL
FIG.6c VIDEO SIGNAL
FIG.6d ADJUSTING SIG. OF INTENSITY OF ILLUMINATION
FIG.6e DELAYED SIGNAL
FIG.6f ILLUMINATING LIGHT

FIG. 7

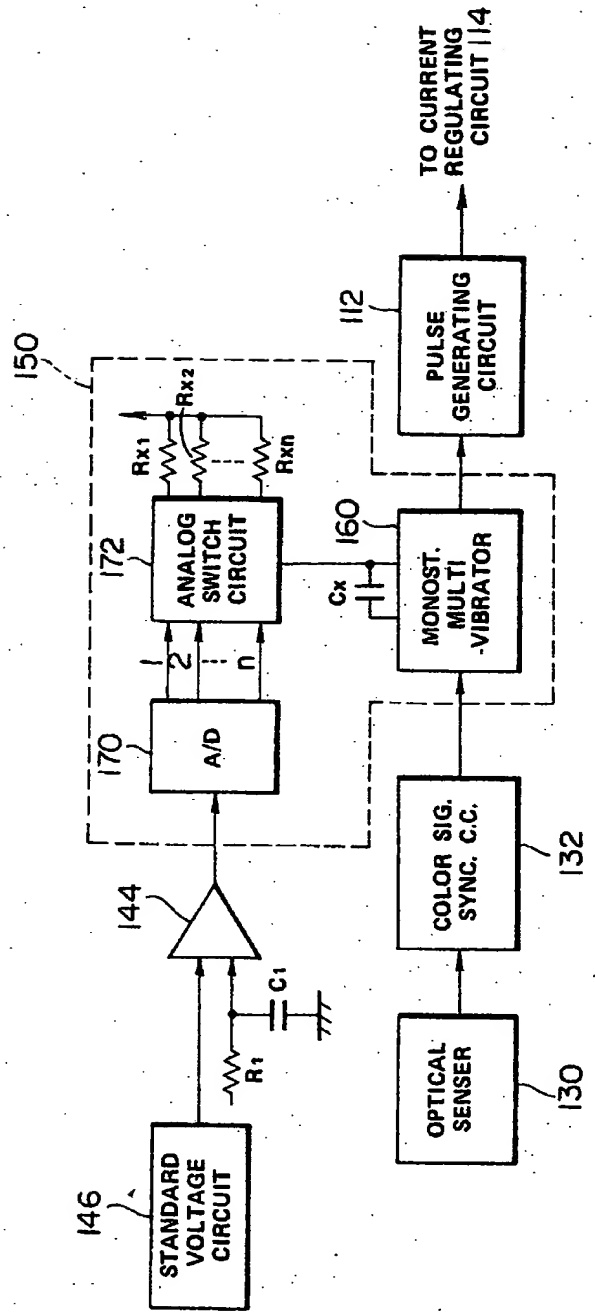

TO CURRENT REGULATING CIRCUIT 114
PULSE GENERATING CIRCUIT
112
150
Rx1
Rx2
Rxn
ANALOG SWITCH CIRCUIT
172
160
Cx
MONOST. MULTI -VIBRATOR
1
2
n
A/D
170
144
C1
R1
COLOR SIG. SYNC. C.C.
132
OPTICAL SENSER
130
STANDARD VOLTAGE CIRCUIT
146

FIG.8

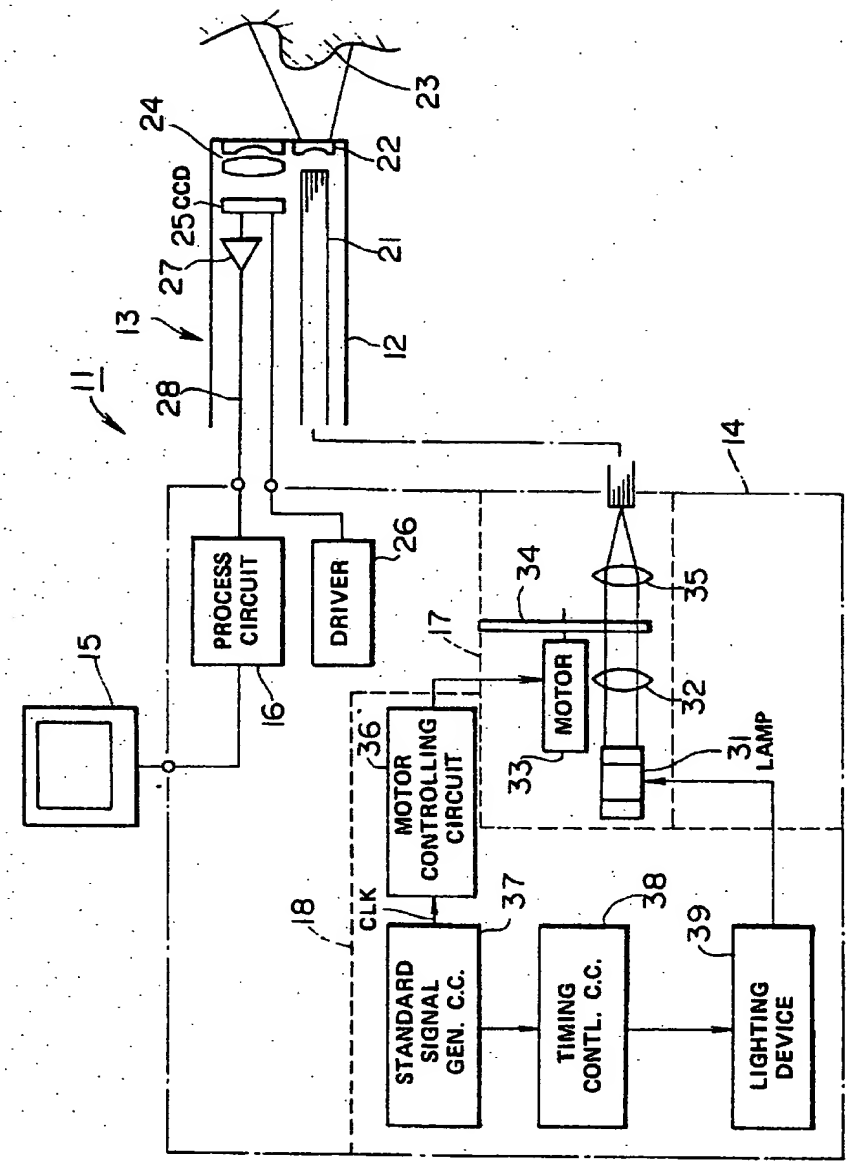

23
24
22
25 CCD
27
21
13
12
11
28
14
PROCESS CIRCUIT
DRIVER
26
34
35
15
17
MOTOR
32
16
36
MOTOR CONTROLLING CIRCUIT
33
31 LAMP
18
CLK
37
38
39
STANDARD SIGNAL GEN. C.C.
TIMING CONTL. C.C.
LIGHTING DEVICE

FIG. 9

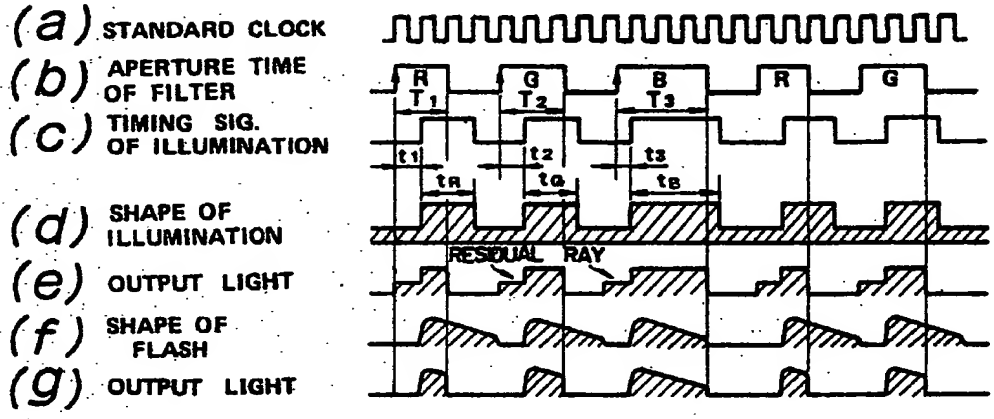

(a) STANDARD CLOCK
(b) APERTURE TIME OF FILTER
(c) TIMING SIG. OF ILLUMINATION
(d) SHAPE OF ILLUMINATION
(e) OUTPUT LIGHT
(f) SHAPE OF FLASH
(g) OUTPUT LIGHT
R T1
G T2
B T3
R
G
t1
t2
t3
tR
tG
tB
RESIDUAL RAY

FIG. 10

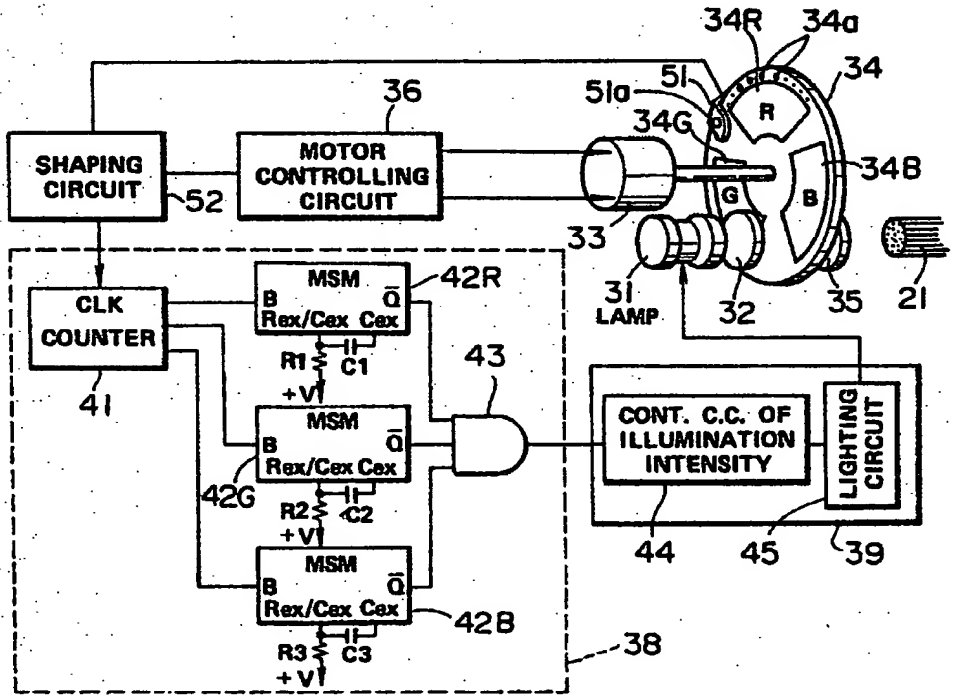

34R
34a
34
51
51a
36
34G
34B
SHAPING CIRCUIT
52
MOTOR CONTROLLING CIRCUIT
R
G
B
33
31
LAMP
32
35
21
CLK COUNTER
41
MSM
B
Q̄
Rex/Cex Cex
42R
R1
C1
+V
43
42G
R2
C2
42B
R3
C3
38
CONT. C.C. OF ILLUMINATION INTENSITY
LIGHTING CIRCUIT
44
45
39

FIG. 11

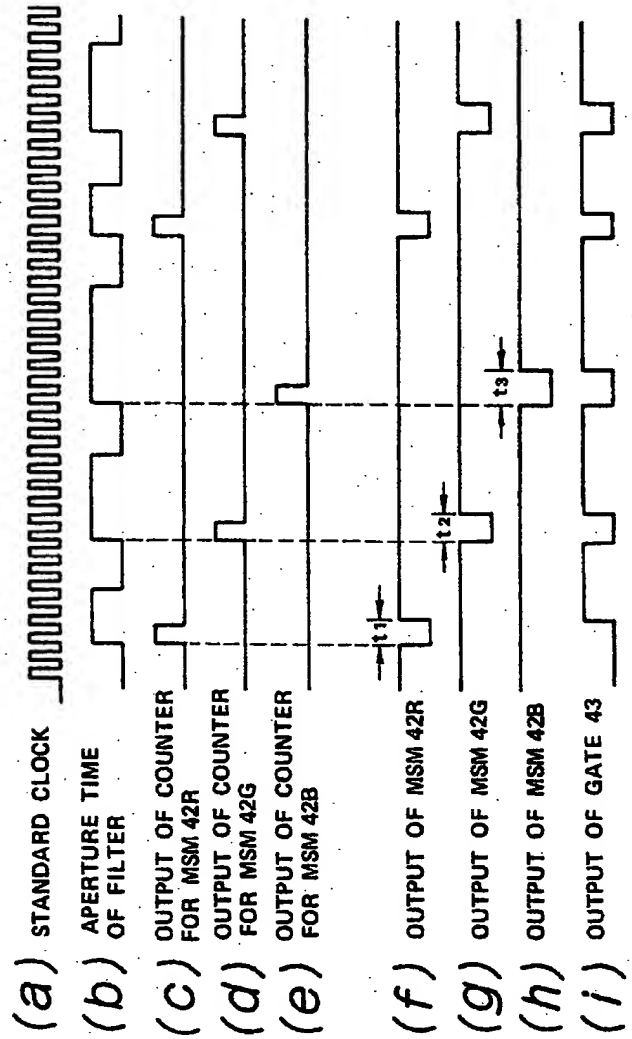
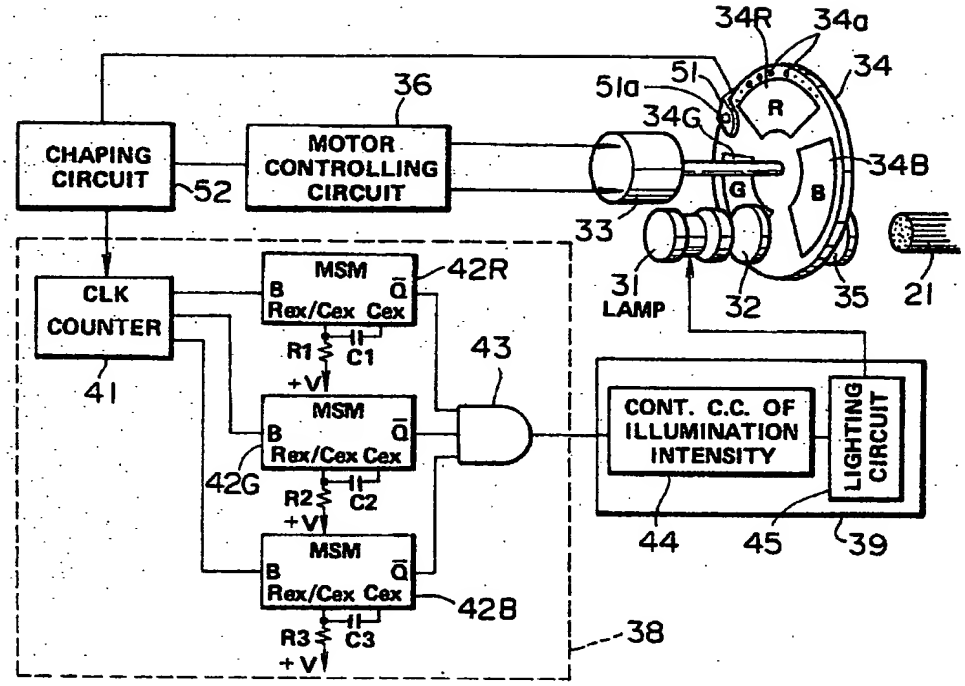

(a) STANDARD CLOCK
(b) APERTURE TIME OF FILTER
(c) OUTPUT OF COUNTER FOR MSM 42R
(d) OUTPUT OF COUNTER FOR MSM 42G
(e) OUTPUT OF COUNTER FOR MSM 42B
(f) OUTPUT OF MSM 42R
(g) OUTPUT OF MSM 42G
(h) OUTPUT OF MSM 42B
(i) OUTPUT OF GATE 43
t1
t2
t3

FIG. 12


34R
34a
34
51
51a
34G
R
G
B
34B
36
CHAPING CIRCUIT
52
MOTOR CONTROLLING CIRCUIT
33
31
LAMP
32
35
21
CLK COUNTER
41
MSM
B
Q
Rex/Cex Cex
42R
R1
C1
+V
43
42G
R2
C2
42B
R3
C3
38
CONT. C.C. OF ILLUMINATION INTENSITY
LIGHTING CIRCUIT
44
45
39

LIGHT SOURCE DEVICE FOR AN ENDOSCOPE WHICH MAINTAINS A CONSTANT MINIMUM-DC CURRENT

This is a divisional application of U.S. Pat. No. 4,800,424.

BACKGROUND OF THE INVENTION

Field of the Invention and Related Art Statement

This invention relates to an endoscope light source apparatus provided with a means of controlling an illuminating light amount to an object to be imaged.

In an endoscopic observation, an exclusive light source apparatus is used so that an illuminating light from the light source apparatus may be radiated onto an object to be imaged from a tip of an endoscope through light guide fibers within the endoscope. In order to adjust the illuminating light amount in response to the brightness (reflection factor) of the object to be imaged, a diaphragm blade is provided between a connector with the endoscope and a lamp within the light source apparatus. A slitted plate or honeycomb plate provided rotatably with the axis intersecting at right angles with a light path as a center is used for such diaphragm blade. That is to say, by electrically or mechanically rotating this a diaphragm blade, the light intercepting rate can be varied to adjust the light amount incident upon the light guide. A related art example using a honeycomb diaphragm is mentioned in a Japanese patent application No. 173832/ 1984.

The endoscope light source apparatus of the above mentioned ralated art example shall be explained with reference to FIG. 1. Here, a so-called electronscope in which a solid state imagining device is built within the tip part of an insertable part to image an object shall be explained. A light source apparatus **102** is connected to an electronscope **100**. The electronscope **100** is provided with a light guide **106** consisting of an optical fiber bundle leading an illuminating light radiated from the light source apparatus to the tip of the insertable part to illuminate an object **104** and a charge coupled device (CCD) **108** as a solid state imaging device built within the tip part.

The light source apparatus **102** is provided with a light source such as, for example, a lamp (xenone lamp) **110**. The lamp **110** is regulated to be of a constant current so that, when an output signal of a pulse generating circuit **112** is input into a current regulating circuit **114**, a flash will be emitted as synchronized with the signal. A switching circuit **116** is connected to the current regulating circuit **114** to form a light source lighting circuit **118**.

The light emitted from the lamp **110** is incident upon the light guide **106** of the electronscope **100** through a diaphragm blade **120**, optical lens system **122** and rotary filter **124**. The diaphragm blade **120** is rotated by a driving device **126** and consists of a slitted plate or honeycomb plate variable in the inclination with the light path. The rotary filter **124** is rotated by a motor **128** and colors the illuminating light in red (R), green (G) and blue (B) in turn. There is a light intercepting period between the exposure periods of the respective color components. An optical sensor **130**, detecting the exposure periods of the respective color components of the rotary filter **124**, is provided. When the exposure periods of the respective color components end, a pulse will be output from the optical sensor **130** and will be fed to a color signal synchronizing circuit **132**. After a predetermined period (corresponding to the light intercepting period) from this synchronizing pulse, only for a fixed period (corresponding to the exposure period), the color signal synchronizing circuit **132** feeds a color synchronizing signal to the pulse generating circuit **112** so that the lamp **110** may be synchronized with the rotation of the rotary filter **124** and may emit a light only in the exposure periods of the respective colors.

The output signal of the CCD **108** is fed to the light source device **102** side through a signal line and connector **138** within the electronscope **100** and is input into a signal processing circuit **140** making an amplification, clamping and various corrections. The output of the signal processing circuit **140** is fed to a video circuit **142** and a picture image is output in a displaying part (not illustrated). The output of the signal processing circuit **140** is input also into a differential amplifier **144**. The standard signal of the differential amplifier is given by a standard voltage circuit **146**. The output of the differential amplifier **144** regulates the diaphragm driving device **126**.

An integrator is also provided within the signal processing circuit so that the illuminating light amount may be detected. The difference between this light amount value and the standard signal is operated by the differential amplifier **144**. In response to this result, the diaphragm driving device **126** is regulated. Thereby, the diaphragm blade **120** will be inclined in the direction of intercepting the light in case the illuminating light is too bright and will be inclined in the direction of opening the light path in case the illuminating light is too dark. This automatic light adjusting operation is the same not only in an electronscope but also in an ordinary fiberscope.

In a conventional light source apparatus for endoscopes, the diaphragm blade is exclusively for adjusting the light, the driving mechanism is for rotating the diaphragm blade and their control circuit are separately required. Therefore, defects have caused the number of the component parts to increase and has caused the light source apparatus to become large, complicated and costly.

Now, in an electronic endoscope apparatus using a field sequential system, in order to improve the color reproductivity of an endoscope picture image, it is necessary to adjust the color balance (white balance) of R, G and B in advance. In the case of using, for example, as a prior art example, the rotary filter 2 provided with respective R, G and B color transmitting filters 1R, 1G and 1B as shown in FIG. 2 for a light source emitting a continuous light, there is used a method of adjusting the aperture ratio of the respective R, G and B color transmitting filters 1R, 1G and 1B by using the light intercepting member 3. Even if the aperture rates of the respective R, G and B color transmitting filters 1R, 1G and 1B are set in advance, depending on the dispersion of the imaging device or the like, it can not be said that the best state can always be held. In such a case, a light intercepting member is further added to the aperture surface of the filter set in advance.

In this prior art example, even if the aperture rates of the respective color transmitting filters are set in advance, due to the dispersion of the imaging device or the like, the aperture rates of the respective color filters must be readjusted by using a light intercepting member. In such a case, defects occur where no accurate

color balance adjustment can be made, a light intercepting member is required and a large amount of time is needed for the adjustment.

When a flash light source is used (which shall be referred to as a strobo lamp hereinafter), and when the energy fed to the strobo lamp is made variable, the emitted light amount can be adjusted. Therefore, when proper energy is fed to the strobo lamp to emit a light within the aperture time of the respective R, G and B color filters as shown in FIG. 3*a*, the R, G and B color balance can be adjusted the same as in the above mentioned example.

In order to make the fed energy variable, there is adopted a method wherein the number of times of the strobo light emission is made variable during the aperture time of the respective color filters.

In the method wherein the number of times of the light emission of the strobo lamp during the aperture periods of the respective color filters is thus controlled, it is necessary to expand the control range by increasing the number of times of the light emission of the strobo lamp which therefore causes a defect in that the life of the strobo lamp is extremely reduced.

Also, as disclosed in a Japanese patent laid open No. 205884/1984 (or U.S. Pat. No. 4,532,918), a multi-strobo-type lamp is used such as the lamp is turned on and off repeatedly. This type of lamp requires a high lighting voltage to be supplied each time it is turned on after a stop. In consequence, noise is liable to be generated in the circuit and the circuit is inevitably rendered complicated in construction.

Objects and Summary of the Invention:

To provide an improved endoscope light source device which is to suppress generation of noise when it is started and which does not require the circuit to become complicated.

In contrast to the multi-strobo-type lamp used in the art of U.S. Pat. No. 4,532,918, the D.C. lighting type lamp used in the present invention can be turned on and off simply by increasing or decreasing the level of the electric current. Namely, it is not necessary to repeatedly turn on and off the high lighting voltage and the generation of noise is suppressed accordingly. The lamp of the type used in the art of U.S. Pat. No. 4,532,918 cannot be used as the light source of the fiber scope, whereas the lamp of the type used in the present invention can be used as a light source for a fiber scope because it can be continously maintained in an on state.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of a light source apparatus of the first prior art example.

FIG. 2 is a view showing a rotary filter used in the second prior art example.

FIG. 3 shows operation explaining diagrams of the third prior art example.

FIG. 4 is a schematic diagram of a light source apparatus of the first embodiment of the present invention.

FIG. 5 is a block diagram showing a detailed formation of the first embodiment.

FIGS. 6*a*–6*f* are timing charts showing the operation of the first embodiment.

FIG. 7 is a block diagram of an essential part of the second embodiment of the present invention.

FIGS. 8 to 11 relate to the third embodiment of the present invention.

FIG. 8 is a formation diagram of an electronic endoscope apparatus provided with the third embodiment.

FIGS. 9(*a*)–(*g*) are an operation explaining view of the first embodiment.

FIG. 10 is a formation diagram of the fourth embodiment of the present invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

FIG. 4 is a schematic diagram of the first embodiment. The same reference numerals are attached to the same parts as in the prior art example in FIG. 1. The difference from the prior art example is that a delaying circuit **150** is provided between the differential amplifier **144** and pulse generating circuit **112** without providing a diaphragm blade and diaphragm driving device. For the convenience of explanation, here the signal processing circuit **140** and differential amplifier **144** are combined to be a light adjusting signal generating circuit **152**.

FIG. 5 shows the details of the delaying circuit **150** and light adjusting signal generating circuit **152** of the first embodiment. The output of the CCD **108** is input into the signal processing circuit **140**. The output of the signal processing circuit **140** is fed to the integrator **154** consisting of a switch S1, resistance R1 and capacitor C1. The switch S1 is controlled to open and close by the control circuit **156**. The output of the integrator **154** is fed to the differential amplifier **144**.

The delaying circuit **150** comprises a voltage controlling resistor (VCR) **158** and a monostable multivibrator **160**. The VCR **158** varies the resistance value with the applied voltage. The output of the differential amplifier **144** is applied to the VCR **158**. The VCR **158** becomes a resistance determining the time constant of the monostable multivibrator **160**. The output of the color signal synchronizing circuit **132** is input into the monostable multivibrator **160** and the output of the momostable multivibrator **160** is fed to the pulse generating circuit **112**.

The operation of the first embodiment shall be explained in the following with reference to the timing charts shown in FIGS. 6*a* to 6*f*. With the rotation of the rotary filter **124**, a color synchronizing signal such as is shown in FIG. 6*a* will be output from the color signal synchronizing circuit **132**. The filtering manner by the rotary filter **124** is shown in FIG. 6*b*. The exposure period is represented by t1 and the light intercepting period is represented by t2. That is to say, when the exposure of the respective colors ends, a color synchronizing signal will be output. The color synchronizing signal triggers the monostable multivibrator **160** but, in this embodiment, the time constant of the monostable multivibrator **160** is determined by the VCR **158** varying the resistance value with the output of the light adjusting signal generating circuit. After the monostable multivibrator **160** is triggered and the time of the time constant passes, the output rises to output a pulse of a fixed width. The resistance value of the VCR **158** will increase when the light adjusting signal increases but will decrease when it decreases. Therefore, the delayed time of the delaying circuit **150** will also increase when the light adjusting signal increases but will decrease when it decreases.

It is assumed that the brightness of an object to be imaged varies from being medium to being small and from being small to being large. The output video signal of the CCD **108** then is shown in FIG. 6*c*. This video signal is integrated by the integrator **154** and the integrated value per frame is held by the capacitor C1. This

integrated output is shown in FIG. 6*d*. The integrated output is fed to one input terminal of the differential amplifier **144**. The standard signal fed to the other input terminal of the differential amplifier **144** is shown by the broken line in the same diagram. The difference between the standard signal and integrated signal is applied as a light adjusting signal to the VCR **158** as a control voltage. That is to say, the larger the brightness of the object to be imaged, the larger the delayed time of the delaying circuit **150**.

Thus, the delayed signal delayed by T in response to the brightness of the object from the color synchronizing signal (in FIG. 6*a*) is fed to the pulse generating circuit **112** from the monostable multivibrator **160**. The delayed signal is shown in FIG. 6*e*. During the period t3 in which this delayed signal is being generated, the pulse generating circuit **112** will flash the lamp **110** through the light source lighting device **118**. Here, when the brightness of the object is medium or large, before the flashing period t3 ends, the light intercepting period t2 of the rotary filter will start and therefore the later half of the emitted light will be intercepted as shown in FIG. 6*f*. Therefore, the emitted light amount of the lamp **110** incident upon the light guide **106** will reduce to be smaller then when the brightness of the object is small. Therefore, even if no diaphragm is used, the light will be able to be automatically adjusted.

The following relations are necessary among the flashing period t3, exposure period t1 and light intercepting period t2.

$$t3<t2 \quad (1)$$

$$t1+t2>T+t3 \quad (2)$$

As explained above, according to this embodiment, there can be realized an endoscope light source apparatus wherein, by delaying the flashing starting timing of the lamp to be later than the exposure starting timing of the rotary filter in response to the light adjusting signal, the light can be automatically adjusted with a simple formation without using the diaphragm blade.

Since as the light adjusting signal is determined on the basis of the difference between the integrated output and standard signal, the light can be adjusted to be of any desired brightness by varying the voltage of the standard signal.

The second embodiment of this invention shall be explained in the following. FIG. 7 is a block diagram of an essential part of the second embodiment. The second embodiment is different from the first embodiment only in the formation of the delaying circuit **150** but is the same otherwise. That is to say, the output of the differential amplifier **144** is input into an A/D converter **170**. The A/D converter **170** converts the A/D by a sampling pulse from the control circuit **156**, makes only the output of any of 1 to n effective and conducts only the corresponding analogue switch in the analogue switch circuit **172**. Thereby, any one of external resistances R×1 to R×n is selected and is connected to the time constant circuit of the monostable multivibrator **160** and the time constant is determined by Rx and Cx. Therefore, also in the second embodiment, as the delaying circuit **150** feeds a delayed signal delayed from the color synchronizing signal to the pulse generating circuit in response to the light adjusting signal, the flashing start of the lamp **110** will be delayed and the light will be automatically adjusted.

This invention is not limited to the above described embodiment and can be variously modified. The lamp **110** may be any flashable lamps such as a direct current arc discharge lamp and strobo lamp. This flashing means that the light amount can be increased or decreased by the increase or decrease of the lamp current. The light need not always be extingushed during the light intercepting period. Further, the delayed time of the delaying circuit **150** can be manually adjusted not by the light adjusting signal and the light may be adjusted manually not automatically. The light source for electronscopes has been explained but can be applied also to a light source for fiberscopes wherein a television camera is fitted to an eyepiece part.

As explained above, according to the first and second embodiments, there can be realized an endoscope light source apparatus of a simple formation requiring neither mechanical diaphragm blade nor diaphragm driving device. Thus, the number of the component parts is reduced, the apparatus can be made small and the price can be made low.

As shown in FIG. 8, an electronic endoscope apparatus **11** provided with the third embodiment comprises an electronic endoscope **13** having an insertable part **12** elongated so as to be insertable into a body cavity or the like, a video processor **14** into which the signal imaged by this electronic endoscope **13** is to be input and a color monitor **15** color-displaying the video signal processed by this video processor **14**.

A (video) processing circuit **16** processing the signal, a light source apparatus **17** feeding an illuminating light and a color balance adjusting part **18** are built within the above mentioned video processor **14**.

A light guide **21** transmitting the illuminating light is inserted through the insertable part **12** of the above mentioned electronic endoscope so that the illuminating light fed from the light source part **17** to the entrance end may be transmitted to be emitted from the exit end and an object **23** to be imaged may be illuminated by the illuminating light expanded through a light distributing lens **22**.

The illuminated object **23** is imaged on the imaging surface of a CCD **25** by an objective lens **24**. When a driving signal output from a driver **26** is applied, the CCD **25** will output a photoelectrically converted signal. This signal is amplified by an amplifier **27** and is then input into the processing circuit **16** within the video processor **14** through a signal cable **28**. The signal is processed by this processing circuit **16** to produce an NTSC compound video signal which is color-displayed by the color monitor **15**.

The above mentioned light source apparatus **17** within the above mentioned video processor **14** is of a field sequential type. The white light of a light source lamp **31** is made a parallel beam by a collimator lens **32**. A rotary filter **34** rotated and driven by a motor **33** is interposed on a light path made by this parallel beam. The light passed through this rotary filter **34** is further condensed by a condenser lens **35** and is radiated onto the entrance end surface of the light guide **21**.

In the above mentioned rotary filter **34**, three sector apertures are formed in a disc frame and color transmitting filters **34R**, **34G** and **34B** transmitting respectively red, green and blue colors are fitted in the respective apertures. When the rotary filter **34** is rotated and driven, these color (transmitting) filters **34R**, **34G** and **34B** will be interposed and retreated sequentially in the course of the light path. The time when the respective

color filters 34R, 34G and 34B are interposed on the light path is mentioned as the aperture time of the filter.

When the above mentioned color filters 34R, 34G and 34B are interposed sequentially on the light path, the lights, that is, the red, green and blue illuminating lights having passed through these color filters 34R, 34G and 34B, will be fed sequentially onto the entrance end surface of the light guide 21. Therefore, the object 23 is illuminated sequentially by the lights of wavelengths of red, green and blue. The signal imaged under this field sequential illuminating state is input into the processing circuit 16.

Now, the motor 33, to make the above mentioned field sequential illumination, is controlled by the rotation controlling signal output from a motor controlling circuit 36 so that its rotating speed may be constant. A standard clock signal CLK output from a standard signal generating circuit 37 is input into this motor controlling circuit 36 to produce a rotation controlling signal synchronized with this standard clock signal CLK. As the motor 33 is synchronized with the standard clock signal CLK and is controlled to be at a constant speed, as shown in FIG. 9*b*, the aperture time when the respective color filters 34R, 34G and 34B are interposed on the light path will be also synchronized with the standard clock signal CLK in FIG. 9*a*. The above mentioned standard signal generating circuit 37 outputs the standard clock signal CLK also to a timing controlling circuit 38. This timing controlling circuit 38 outputs a light emitting timing signal such as is shown in FIG. 9*c* to a lighting device 39 lighting the light source lamp 31 to control the light emitting time of the light source lamp 31.

By the above mentioned light emitting timing signal, the light emitting time of the light source lamp 31 is controlled and, by the relation between this light emitting time and the filter aperture time, the fed light amount (emitted light amount) fed actually to the light guide 21 is variably controlled and the color balance is adjusted.

That is to say, when the light emitting timing signal is made to coincide with the aperture time of the filter, the timing when the color filters 34R, 34G and 34B are interposed on the light path and the timing when the light source lamp 31 emits the light will coincide with each other and therefore the emitted light amount to the light guide 21 will become large. On the other hand, when the light emitting timing time is displaced as shown in FIG. 9*c* from this filter aperture time, as shown in FIG. 9*d*, the light emitting time will be displaced and the light emitting waveform will be also displaced. Only for the light emitting period overlapping the aperture time, the output light will be fed actually to the light guide 21. This output light period is shown in FIG. 9*c*.

Thus, the timing of outputting the light emitting timing signal is controlled and the emitted light amount to the light guide is variably controlled to adjust the color balance.

The operation of the embodiment in FIG. 7 shall be explained in the following with reference to FIG. 9.

As in FIG. 9*a*, a standard clock signal is oscillated by the standard signal generating circuit 37 and is input into the motor controlling circuit 36 and timing controlling circuit 38. The rotary filter 34 fitted to the motor 33 is controlled to rotate at a constant speed by the motor controlling circuit 36 by the signal synchronized with the standard clock signal. Therefore, as in FIG. 9*b*, the aperture time and timing of the respective color filters synchronized with the standard clock signal are determined. Here, T1 represents the aperture time of R(red), T2 represents the aperture time of G(green) and T3 represents the aperture time of B(blue). As in FIG. 9*c*, a light emitting timing signal synchronized with the standard clock signal, the same as in the above mentioned aperture time, is output from the timing controlling circuit 38. Signals delayed by the time t1 for the rise of the aperture time of R, by the time t2 for the rise of the aperture time of G and by the time t3 for the rise of the aperture time of B are shown to be output. The signal shown in FIG. 9*c* is input into a lighting device 39 for lighting and controlling the light source lamp 31. The light source lamp 31 emits a light as synchronized with the above mentioned light emitting timing signal. FIGS. 9*d* and 9*e* are of examples of controlling the current fed to a continuously lighted lamp such as a direct current arc discharge lamp. The output light output on a low level shows a residual light. In this example, the current of the lamp is controlled as synchronized with the light emitting timing signal to control the emitted light.

As shown in FIG. 9, as the light source emits a light (the light amount increases) as delayed by the time t1 (or t2 or t3) for the timing of the filter aperture time T1 (or T2 or T3), the light emission will continue to the light intercepting period of the rotary filter 34. That is to say, if the filter aperture time and the light emitting timing are simultaneous without delay, the emitted light will be all emitted during the period of tR (or tG or tB) and the light amount will be 100% output light but, if a delayed time of t1 (or t2 or t3) is provided, the emitted light will be delayed by the time of t1(or t2 or t3) and the light will become darker by the emitted light for the time of t1 (or t2 or t3) than in the state of 100%. In the present application, the color balance is adjusted by utilizing this point. When the above mentioned time t1, t2 and t3 are independently freely controlled, the amount of the emitted light colored in the respective colors will be varied and therefore the color balance can be adjusted very simply and accurately. For example, after the time t1, t2 and t3 are set to be optimum, if the time t1, t2 and t3 are varied at the same ratio so as to follow the brightness level of the video signal, the light can be adjusted.

FIGS. 9*f* and 9*g* are of examples of using a strobo lamp. The difference in this case is the difference of the light emitting system of the light source lamp 31.

The above mentioned direct current arc discharge lamp has a characteristic that it can not be lighted just after it is extinguished. Therefore, the lamp current is increased or decreased to emit a light as synchronized with the light emitting timing. In such a case, a residual light shown in FIG. 9*e* will be generated. On the other hand, in the strobo lamp, there are advantages that the light can be perfectly set on and off, can be therefore continuously emitted within a short time, the flashing time is also short, therefore the imaging time is short and a sharper image is obtained.

FIG. 10 shows a concrete formation of the fourth embodiment.

In this embodiment a sensor 51 is for sensing the rotating speed of the rotary filter 34 and the waveform shaping circuit 52 is for shaping the waveform of a signal output from this sensor 51 and inputting the signal into the counter 41.

The above mentioned sensor 51 comprises a light emitting device and light receiving device 51*a* arranged

to hold a filter frame, for example, of the rotary filter 34 so that a light pulse, in case the light receiving device 51a receives the light of the light emitting device through holes 34a provided at regular intervals in the peripheral direction of the filter frame, may be photoelectrically converted and led to the waveform shaping circuit 52.

The motor 33 fitted with the rotary filter 34 rotates at a constant speed by the motor controlling circuit 36. The sensor 51, sensing the rotating speed of the rotary filter 34, is provided in a proper position on the periphery of the rotary filter 34 so that the signal obtained from the sensor 51 may have the waveform shaped by the waveform shaping circuit 52. This signal operates as a standard clock signal as in FIG. 9a.

The operation of this embodiment shall be explained in the following.

A standard clock signal obtained by the above mentioned waveform shaping circuit 52 is counted by the counter 41, is synchronized with the aperture time of the filter and is fed to the respective delaying circuits 42R, 42G and 42B. Therefore, a light emitting timing signal, having the delayed time t1, t2 and t3 set by the constants Ri and Ci provided in the respective delaying circuits, is fed to the lighting device 39 as in FIG. 9c. Therefore, the emitted light amount passing through the respective color filters can be freely adjusted and therefore the color balance can be adjusted.

In this embodiment, even when the speed of the rotary filter 34 is unstable, as the rotating speed is always sensed and the light emitting timing signal is output, the color balance can be adjusted more accurately.

A means of detecting the positions of the respective color filters interposed on the light path may be provided and the light may be extinguished except at the aperture time.

The above mentioned respective embodiments have a feature that the colors can be balanced irrespective of the kind of the light source lamp.

The emitted light amount can be also controlled by emitting, as pulses, the emitted light amounts of the R, G and B lamps.

As described above, according to the fourth embodiment, as the lighting of the light source lamp is controlled through the delaying means synchronized with the time when the respective color filters of the rotary filter are interposed, the color balance can be adjusted at a high precision with a simple formation.

What is claimed is:

1. A light source device for supplying illuminating light to an endoscope comprising:
- a short-arc lamp emitting light in response to a supply of a D.C. current;
- lighting circuit means for supplying a D.C. base current of a low level to said short-arc lamp and for supplying a pulse current of a high level at a suitable period to turn on said short-arc lamp; and
- shutter means, disposed in a path of light of the illuminating light emitted from said short-arc lamp, said shutter means for opening and closing at a period which is substantially the same as the period of turning on of said short-arc lamp.

2. A light source device according to claim 1, wherein said lighting circuit means is for supplying at least said D.C. base current in the period in which said shutter means is closed.

3. A light source device according to claims 1 or 2, wherein said lighting circuit means is for supplying said D.C. base current regardless of a state of said shutter means.

4. A light source device according to any one of claims 1 or 2, wherein said shutter means includes a rotary filter having a light-shielding disk frame having a plurality of sector openings arranged in the circumferential direction and color transmitting filters on the plurality of sector openings, and a motor for rotatingly driving said rotary filter to bring said color transmission filters successively into the path of the illuminating light.

5. A light source device according to claim 4, wherein said color transmission filters transmit lights of wavelengths corresponding to red, green and blue colors.

6. A light source device for supplying illuminating light to an endoscope comprising:
- a short-arc lamp emitting light in response to a supply of a D.C. current;
- lighting circuit means for supplying a D.C. base current of a low level to said short-arc lamp and for supplying a pulse current of a high level at a suitable period to turn on said short-arc lamp; and
- shutter means, disposed in a path of light of the illuminating light emitted from said short-arc lamp, said shutter means for opening and closing at a period which is substantially the same as the period of turning on of said short-arc lamp, said shutter means including a rotary filter having a light-shielding disk frame having a plurality of sector openings arranged in the circumferential direction and color transmitting filters on the plurality of sector openings, and a motor for rotatingly driving said rotary filter to bring said color transmission filters successively into the path of the illuminating light, wherein said lighting circuit supplying said D.C. base current regardless of a state of said shutter means.

7. A light source device for supplying illuminating light to an endoscope comprising:
- a short-arc lamp emitting light in response to a supply of a D.C. current;
- lighting circuit means for supplying a D.C. base current of a low level to said short-arc lamp and for supplying a pulse current of a high level at a suitable period to turn on said short-arc lamp; and
- shutter means, disposed in a path of light of the illuminating light emitted from said short-arc lamp, said shutter means for opening and closing at a period which is substantially the same as the period of turning on of said short-arc lamp, said shutter means including a rotary filter having a light-shielding disk frame having a plurality of sector openings arranged in the circumferential direction and color transmitting filters on the plurality of sector openings, and a motor for rotatingly driving said rotary filter to bring said color transmission filters successively into the path of the illuminating light, wherein said lighting circuit means for supplying at least said D.C. base current in the period in which said shutter means is closed.

8. A light source device according to claims 6 or 7, wherein said color transmission filters transmit lights of wavelengths corresponding to red, green and blue colors.

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